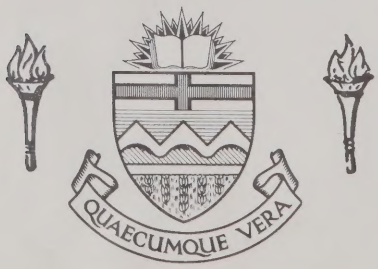


The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

PERSISTENCE AND FUNCTION OF THE BURSA OF FABRICIUS: A PRIMARY LYMPHOID ORGAN OF THE CHICKEN

by

© Peter Ross McConnachie

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA

SPRING, 1970

ABSTRACT

This study is based on evidence that the bursa of Fabricius plays an important role in the development of immune competence. The bursa has two distinct components, epithelium and lymphocytes. The lymphoid tissue regresses and the epithelium may, or may not persist a long time. The basic question of this study was simply, how long does the epithelium persist? Ancillary questions were, does the epithelium function specifically in the early part of life, and particularly in the later part of life, if so, how?

Bursa epithelium was found in the oldest birds examined, in some birds it was not. A relationship was demonstrated between survival and persistence of the epithelium which was interpreted to mean that epithelial persistence enhances survival.

A possible functional activity of persisting epithelium was indicated by results of light and electron microscope studies, histochemical studies, innervation studies and RNA and DNA precursor uptake studies.

A demonstration of specific epithelial function in the development of immune competence was attempted through bursectomy and replacement experiments. Neonatal

surgical bursectomy inhibited or delayed antibody production and increased mortality. Immediate replacement of extirpated bursas significantly restored antibody production and significantly reduced mortality. A clear relation of neonatal epithelium to the restoration was not demonstrated. The results do not preclude the implied relation.

Entirely different effects were obtained by surgical bursectomy of adult bursas undergoing lymphoid regression. These were a significant increase in the magnitude of variation of peripheral leucocyte counts and a tendency towards enlargement of other lymphoid organs. The increased magnitude of variation of leucocyte counts in bursectomized birds was evident in two ways. There was greater variation in bursectomized counts in comparison to unoperated and sham operated counts at each weekly measurement and also in terms of the increase or decrease of peripheral leucocytes from week to week. The tendency toward other lymphoid organ enlargement was not uniform in both sexes. Spleen enlargement was observed in individuals of both sexes but thymic cortex enlargement was demonstrated only in females. The suggested explanation of these effects is the involvement of the adult bursa and, by implication, the persisting epithelium of the bursa, with spleen and thymus in the maintenance of the lymphoid system.

ACKNOWLEDGMENTS

Financial support was provided by National Research Council Scholarships and National Research Council and Medical Research Council of Canada grants in aid of research to Dr. R. F. Ruth. The experimental animals were obtained through the University of Alberta Poultry Farms and from stock provided by Hyline Poultry Farms, Johnston, Iowa. Facilities for ultrastructure studies were provided by the Electron Microscope Laboratory.

I wish to thank Dr. R. F. Ruth for the continued stimulus, discussion and support which made this study possible. I wish to thank my wife for her graceful perseverence.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

The bursa of Fabricius and thymus are the primary lymphoid organs of birds. These appear as epithelial rudiments during the fifth day of embryonic life. Lymphoid differentiation begins at seven and one half days in the thymus (Ackerman and Knouff, 1963) and during the thirteenth day in the bursa (Meyer, Rao and Aspinall, 1959). Thus, the bursa is the second avian lymphoid tissue.

The bursa consists of an endodermal epithelium, lymphoid follicles and a mesenchymal stroma. The epithelium surrounds a central lumen opening into the proctodeal chamber of the cloaca. Longitudinal folds of the epithelium reduce the lumen to a complex of interconnecting slits. Each fold contains many lymphoid follicles which maintain continuity with the epithelium. Between follicles there is a mesenchymal core continuous with an external sheath of mesenchyme. Peritoneum covers the organ. The mature bursa is oval, approximately 3.5 cm. long and 1.5 cm. wide, and points forward in the peritoneal cavity from the posterior dorsal aspect of the cloaca to which it is connected by a stalk.

Surgical expirpation of late embryonic and early neonatal bursae severely impairs or inhibits antibody production and reduces serum immunoglobulin levels, numbers of splenic germinal centres and plasma cells (Cooper, Cain, Van Alten and Good, 1969). Treatment of the embryo with

testosterone inhibits bursa lymphoid development and severely impairs antibody production up to eight months later (Mueller, Wolfe and Meyer, 1960). Surgical bursectomy or testosterone treatment have little or no effect when performed two weeks after hatching or later (Mueller, Wolfe and Cote, 1964, Warner and Burnet, 1960). Thus there is a relationship between the bursa and antibody production but it is subject to manipulation only very early in life.

The bursa does not remain static throughout life. It grows to maximal size by twelve to fourteen weeks and then rapidly regresses to less than 30 per cent of maximal weight by twenty two weeks (Sheridan, 1967). This implies that the bursa has an important role during the development of the immune system but has little to do with homeostasis or maintenance of immune competence in adult life.

Growth and regression of the bursa have been measured by change in weight (Glick, 1956) and thus represent quantitative rather than qualitative changes. This rapid increase and decrease in weight really represents a proliferation of lymphocytes in lymphoid follicles followed by a rapid loss of lymphoid follicles. The regressed bursa is largely an epithelial structure (Jolly, 1915) and the epithelium may not be subject to regression as is the lymphoid tissue of the bursa. Thus, the questions are raised: how long does the epithelium of the bursa persist, does it function in adult life and, if so, how?

The first step in this study is a description of the persistence and character of the bursa in time with particular reference to the epithelium. The second step is an attempt to identify molecular and functional peculiarities of mature and regressing bursae and the third step is an attempt to relate the mature epithelium to immunological function.

PART 1 PERSISTENCE OF THE BURSA OF FABRICIUS

MATERIALS AND METHODS

Persistence of the bursa of Fabricius

In order to assess the disappearance or, conversely, the persistence of the bursa as an organ, I decided to examine Single Comb White Leghorns raised from Hyline Poultry Farm Stock (Hyline Poultry Farm, Johnston, Iowa) because of known data on the regression of bursa weight in White Leghorns (Sheridan, 1967) and, because this stock is under genetic control. Other lines (Black Rock, White Rock and Barred Rock, University of Alberta, Dept. of Poultry Science) were selected simply for their availability.

A total of 138 birds ranging in age from 2 to 54 months were examined histologically to ascertain the presence or absence of the bursa.

Up to 6 months of age, bursas were grossly apparent and were selected for examination as needed. Beyond 6 months of age, bursas were frequently not grossly apparent. Then, the posterior dorsal cloacal area of each bird was dissected out, fixed in formal-Calcium (Barka and Anderson, 1965), gum-sucrose impregnated, cryostat sectioned and stained by a rapid hematoxylin-eosin method (Stevens and Mainwaring, 1967), whether a recognizable bursa remnant was present or not.

Histochemistry and Histology of bursa epithelium

Because of the possibility of modification of the epithelium of the bursa during regression, histochemical studies of enlarging and regressed bursas were carried out as follows:

Azure -A staining (after Szirmai, 1963) with ribose-nuclease, diastase and hyaluronidase digestions (Culling, 1964, Pearse, 1961) to detect protein and polysaccharide components.

Alcian blue-periodic acid Schiff staining (after Scott, Quintarelli and Dellova, 1964), with hyaluronidase and trypsin digestions to detect protein and acidic mucopolysaccharides.

Acridine orange fluorescent staining (Bertalanffy, 1962) with ribosenuclease digestion to detect RNA.

Biebrich Scarlet staining (Spicer and Lillie, 1961) to detect protein.

Naphthol phosphate methods for alkaline and acid phosphatases (Barka and Anderson, 1965) for comparisons with known associations of alkaline phosphatases with lymphoid follicle development (Ackerman and Knouff, 1963) and acid phosphatases with bursa epithelium (Chen, 1968).

Some of the preceding methods were applied to coprodeal and proctodeal epithelia for comparison.

Ultrastructural Studies

Epithelial areas of enlarging and regressing bursas

were fixed, embedded and sectioned for examination with the Phillips 100 B electron microscope after the methods of Luft (1961).

RESULTS

Persistence of the Bursa

The first birds examined were the Black, White and Barred Rocks 12 to 34 months old. The results are shown in Figure 1. A small, epithelial, non-lymphoid bursa was found in 79 per cent of the birds. Most of the birds lacking any detectable bursa remnant were 12-14 months old; only a few were 15 months old.

The data pertaining to the White Leghorns examined is shown in Figure 2. In short, up to 6 months of age all birds have a bursa which is epithelial but decreasingly lymphoid. That is, the size and number of lymphoid follicles decreases. From 8½ months on, only 63 per cent of the White Leghorns examined had an epithelial bursa remnant.

Bursa Persistance and Age

Two striking observations were apparent in the preceding histological survey. Fifteen month old birds had the highest incidence of epithelial non-lymphoid bursa than any other age range in both the White Leghorns and the other breeds examined. This raised the possibility that there might be a relationship between the absence or presence of the bursa and age, detectable through analysis of survival. Accordingly, a survival curve was constructed from mortality

data taken from White Leghorns. These birds were part of the hatches raised for the histological survey, but had not been utilized for that purpose. The survival curve can be separated into three distinct lines according to the regression of per-cent survival on age (Figure 3). This indicated the correct partitioning of the superimposed bursa 'survival' curve (direct transformation of data from Figure 2) for analysis. A chi square test of the three age ranges of proportions of bursa absence or presence was significantly different. This is interpreted to mean that the proportions of birds with and without bursas changes considerably during the three periods of the survival curve. The changes indicate that birds which lose the bursa entirely between 6 and 10 months tend to die during that same period. This would confer considerable survival value on the persistence of the epithelial component of the bursa.

Histology of Regressing bursas

Bursa regression is largely due to a decrease in size of lymphoid follicles beginning at 2½ months of age. By 8 months, lymphoid follicles have decreased in diameter by a factor of 10 to 0.1-0.75 mm. Persistence of lymphoid follicles beyond 8 months is in the form of isolated follicles, 0.1-0.35 mm. in diameter, adjacent to, but not generally within the bursa remnant. Within the 8 month and older remnant there are consistently large areas of lymphocyte-like cells which are located near the lumen epithelium but

lack follicular organization.

Figures 4 through 7 show a month old enlarging bursa, a 15½ month persisting bursa and two intervening stages. Figures 8 and 9 show two extremes in size of persisting bursas. Most bursas which persist are of the same morphology as in Figure 8, about 5-6 mm. long and about 3-4 mm. in diameter. Persisting bursas were sometimes much smaller but rarely any larger.

Histochemistry and Histology of bursa epithelium

Morphological and histochemical studies of enlarging (3 month old) and persisting (13 month old) bursa epithelia, and of 13 month proctodeal and coprodeal epithelia are summarized in Tables I and II. These indicate:

(1) Persisting bursa epithelium is less complex, but similar to enlarging bursa epithelium.

(2) Cloacal epithelia (proctodeal and coprodeal) are morphologically and histochemically distinct from the epithelium of enlarging and persisting bursas.

(3) Ribosenucleic acid and protein which stains at slightly acidic pH are major components of material in 3 month and 13 month epithelia.

(4) An unidentified acidic polysaccharide component is associated with the protein and is localized near the epithelial surface in 3 month and 13 month bursas.

(5) Alkaline phosphatase activity is found just below the epithelium of all ages of bursa examined.

(6) Acid phosphatase activity is found in epithelial cells near the lumen surface in all ages of bursa examined.

Histochemically, within the limits of the tests employed, persisting epithelium is not different from 3 month epithelium at the peak of bursa development.

Figures 10 through 19 illustrate the difference between bursal and cloacal epithelia, some of the histochemical results described and a comparison of enlarging and persisting epithelia.

In some extremely regressed bursas, a new type of epithelium is apparent. Such a bursa was shown in Figure 9 and the epithelium in Figures 20 and 21. The epithelium is cuboidal, approximately 10 to 15 microns in thickness and appears in lymphoid follicles during the process of regression. This epithelium is distinct from persisting epithelium in several characteristics: it lacks associated alkaline phosphatase activity, it has a simple cuboidal cellular structure and it demonstrates very little acid phosphatase activity.

The products of lymphoid follicle degeneration are contained and seem to persist in cysts bounded by this epithelium. For that reason it has been designated cyst epithelium.

I found no bursa remnant in which cyst epithelium was the predominant or only epithelium present. Persisting epithelium is always predominant in a regressed bursa.

Ultrastructural Studies

Ten and Twelve week old Bursa

Sections of epithelium from three different bursas were examined. The epithelium is pseudostratified columnar with a cuboidal organization in the basal area. Desmosomes are common and form terminal bars at the lumen surface between adjacent cells. At this age the typical nucleus is an ellipsoid 7-8 microns long. Nuclei are at 4 or 5 levels. The elongated cells are interdigitated with each other and with the smaller cuboidal cells of the basal layer. Basal cell nuclei are round or oval, 4-5 microns in diameter. Most cells contain large vacuoles of transparent material. Some cells have smaller more dense vacuoles. The vacuoles occupy large areas of the cell adjacent to, or at some distance from the lumen surface. A Golgi apparatus of stacked membrane systems and multivesicular bodies is well developed. Numerous short microvilli, up to 500 millimicrons long and 100 millimicrons wide are irregularly spaced at the apical end of each cell and project into the lumen.

Twenty-six week old Bursa

Sections of epithelium from seven bursas were examined. In contrast to the younger epithelium, only the round or oval nucleus is present. These are at two levels, in a single columnar cell layer and in a basal cell layer, and are 4-5 microns in diameter. Interdigitations, desmos-

omes and terminal bars are seen as in the three month epithelia. Large and small vacuoles containing transparent material are in most cells of the columnar layer and in some of the cells of the basal layer. These vacuoles are more numerous and smaller than in the younger epithelium. Dense vacuoles are larger and more numerous near the lumen surface. Microvilli and Golgi apparatus are not strikingly different than in three month epithelium.

These results indicate that:

(1) Bursa epithelial cells at three months and six or seven months contain similar secretory material.

(2) Epithelial cells of both ages are similar in morphological details of microvilli, terminal bars and interdigitations.

(3) The nuclear morphology, characteristic of the younger epithelium, is not common in the older tissue. The basal layer of the epithelium is similar at both ages.

(4) The architecture of the epithelium changes slightly from three to seven months in that a large elongated cell is not present to the same extent at seven months and the whole epithelium becomes thinner.

The ultrastructure of bursa epithelium I observed was consistent with published observations of bursa epithilium (Cheville, 1967).

PART 2 PHYSIOLOGICAL CHARACTERISTICS OF ENLARGING AND REGRESSING BURSAS

MATERIALS AND METHODS

Innervation

There is little available information regarding the innervation of the avian bursa. To consider the bursa as an organ raises the question: What innervation exists and does it persist? Bursas ranging in age from 20 days embryonic to 15 months were examined for adrenergic nerves by the paraformaldehyde fluorescence method (Falck, 1962) and for cholinergic nerves by the butyryl-thio cholinesterase method (Karnovsky and Roots, 1964). Standard silver impregnation methods and an in vitro methylene blue uptake method (Arthur and Shelley, 1959) were used to detect neurons. As a control the Gomori method for reticulin fibres was applied to some sections.

Radioactive Thymidine and Uridine uptake

Since bursa epithelium persists and protein and RNA are major components of epithelial cells, mitotic and synthetic activities should be detectable in epithelium of all ages.

These activities were examined in bursas taken at 3 to 29 weeks.

The first experiment was short term tissue cul-

ture of fragments of 3 week and 20 week bursas in medium incorporating labelled thymidine or uridine. The isotopes were; uridine -H^3, 24.7 C/mM (New England Nuclear) and thymidine -H^3, 17.5 C/mM (Amersham). The culture medium was Hank's Balanced Salt solution (BSS) 40%, chicken serum 40% and 10 day embryo extract 20% (Parker, 1961). Each culture contained 25 uC of isotope, 100 units/ml. penicillin and 100 ugm./ml. streptomycin. Labelling was for two hours at 40° C. and under 95 per cent oxygen and 5 per cent CO_2. The culture medium was then replaced with fresh medium containing an excess of cold thymidine or uridine. Tissue was then rinsed in BSS five times prior to Bouin fixation and paraffin processing. Some sections were tested with ribosenuclease and all slides were stained with hematoxylin and eosin, air dried and then coated with liquid emulsion. Autoradiographs were made using Kodak NTB 2 liquid emulsion according to methods of Prescott (1964).

The second experiment was designed to afford an <u>in vivo</u> comparison with tissue culture. Tritiated thymidine or uridine was either (a) injected directly into the lumen of surgically exposed but intact bursas of anaesthetized birds or

(b) injected intravenously into the brachial vein and locally into the general area of the bursa in intact birds.

In the first case 50uC of thymidine-H^3 was injected into the bursa of a 13 week old bird and 50 uC of uridine-H^3 into a 13 week and a 29 week bursa. The birds were maintained in this condition for 85 or 90 minutes. The bursas were then excised and processed as described above.

In the second case two 7 month old birds were given isotope as follows:

	Bird #15	Bird #446
	thymidine-H^3	uridine-H^3
intravenous	550uC	650uC
local	200uC	200uC
	750uC	850uC

After 24 hours the birds were sacrificed, the bursas removed and treated as described. RNAase and DNAase digestions were controls.

All autoradiographs were exposed for three weeks, developed in Kodak D-19 developer and fixed. Grain counts per cell were made in epithelium, supportive and lymphoid tissues.

RESULTS

Innervation

Silver impregnations demonstrated neurons in the 20 day embryo bursa and in 6, 15 and 17 month bursas. Comparable sections treated to demonstrate reticulin fibres never displayed a similar distribution of morphology of fibres. The number of demonstrable neurons in the microscopic field increased during lymphoid regression in the bursas treated

with the methylene blue method. Neurons frequently approached the follicular cortex in the growing bursa, but in the regressing bursa they approached the stroma and basal cells immediately beneath the persisting epithelium. Direct contact of neurons and epithelium was observed occasionally. The increased number of demonstrable neurons during regression may be a mechanical redistribution due to actual shrinkage of the bursa.

The Falck method demonstrated adrenergic fibres in 2 day chick bursas apparently in the inter follicular connective tissue. Fibres or neurons as such were not resolved but the flourescence was specific as it was quenched by sodium borohydride treatment and renewed again after reexposure to paraformaldehyde (Corrodi, Jonsson and Malmfors, 1966). In older and regressing bursas adrenergic fibres were demonstrated near epithelium and more frequently just outside the periphery of the bursa.

The methylene blue uptake method was intended as a comparison to the standard silver methods in some older birds. It was by this method that the increased numbers of neurons were demonstrated (Figure 30). Ehinger, Sporrong and Stenevi (1967) suggested that this method may actually be specific for cholinergic fibres. Tests for acetylcholinesterase were always positive but there was a specificity problem in that eserine sulphate inhibition (Karnovsky and Roots, 1964) was not always complete. There were extensive

areas of enzyme activity just beneath the epithelium of 6 month and older birds. This result was consistent, but no fibres were detected. This method was not applied to birds younger than 21 weeks because of the failure to demonstrate cholinergic fibres (by their cholinesterase activity) in those bursas which were examined. The results of the innervation studies could be summarized as follows:

(1) Neurons were found in bursas of all ages examined.

(2) Adrenergic nerve fibres were found in bursas of all ages examined.

(3) Methylene blue detected many fibres but attempts to prove that these were cholinergic failed.

(4) When lymphoid follicles are present, some fibres penetrate the lymphoid cortex; in the absence of follicles some fibres appear to approach very close to the epithelium.

(5) A large ganglion of the autonomic system is close to the base of the bursa.

Some of these results are shown in Figures 28 through 34.

Radioactive thymidine and uridine uptake

The results of the tissue culture experiment with 3 week and 20 week bursa fragments are shown in graphic form in Figure 35. By this method of labelling, isotope incorporation was minimal- and rarely exceeded 3 grain counts/cell. At both ages the incorporation of uridine-H^3 by bursa epith-

elium was three times that of the stroma cortex or medulla. RNAse digestion reduced the number of grains by half but the tissue ratio remained the same.

Thymidine-H^3 incorporation was much less than uridine but epithelial incorporation was slightly more than all other areas of the bursa in both 3 and 20 week tissue fragments. The low incorporation of both labels and the consistent higher epithelial incorporation suggest a surface absorption effect.

The results of the in vivo administration of label are shown in Figure 36. Incorporation in vivo was much more extensive than in culture. The increase cannot be due to difference in amount of label as the time of labelling was less in vivo than in culture. Differences were apparent according to the age of bursas, the route of administration and the particular label given.

In 13 week bursa given intra-bursa uridine-H^3, the epithelium and the sub-epithelial connective tissue (stroma) incorporated up to 5 grains/cell. The cortex of lymphoid follicles adjacent to the epithelium demonstrated double the epithelial activity. The medulla incorporated much less. Most of the thymidine incorporation occurred in the epithelium; the sub-epithelial stroma and follicles were less active. The epithelium which lies at the base of the follicles and is continuous with the medulla did not incorporate thymidine.

Intra-bursal administration of isotope did not produce uniform labelling of tissues. Figure 36B shows two epithelial counts demonstrating a five-fold difference. The high count is from an area close to the point of injection of the isotope, the low count is some distance away (approximately 1 cm.). The short time of labelling did not permit extensive diffusion of label throughout the lumen.

Background activity was negligable.

The incorporation of uridine-H^3 following intra-bursal administration in 28 and 29 week bursas was not different from that in 13 week bursas. Epithelial incorporation at this age was much less when given by the intravenous route. This suggests a non-specific absorption of thymidine or uridine by epithelium when the base is injected into the bursa lumen or provided in tissue culture.

In the 28 and 29 week bursas the cells incorporating thymidine-H^3 are not at the same location as the cells which incorporate at 13 weeks. In the latter, incorporation is primarily nuclear in the basal layer of the epithelium, in the older bursas, fewer cells incorporate more isotope and the cells are distributed in the basal layer of the persisting epithelium and in the sub-epithelial connective tissue.

The few lymphoid follicles in the 28 and 29 week bursas were in the process of regressing. These follicles still demonstrated considerable uridine and thymidine-H^3

incorporation. Cells incorporating thymidine-H^3 were heavily labelled and not distinguishable from the heavily labelled cells near, or within, the epithelium.

These results may be summarized:

(1) RNA synthetic activity in 13 week bursas is highest in the follicular cortex, least in the medulla and intermediate in the epithelium.

(2) RNA synthetic activity in 28 week bursa epithelium is comparable to activity in 13 week epithelium. In 29 week lymphoid areas activity is comparable to that in the 13 week medulla.

(3) Mitotic activity in the 13 week bursa is highest in the basal area of epithelium between follicles. In 28 and 29 week bursas, comparable activity is found in cells generally located in or near the basal layer of the epithelium.

(4) The data may indicate an absorptive capacity of the epithelium of 13 week and 28 or 29 week old bursas and a transport of label or cells from the epithelium to other tissues in the bursa.

These results are illustrated in Figures 37 through 44.

PART 3 THE RELATION OF THE BURSA TO IMMUNOLOGICAL FUNCTION

Neonatal Bursectomy Experiments

MATERIALS AND METHODS

A more suitable system to study the effect of bursectomy and replacement on immune function than had been employed was suggested in 1964 by Mueller, Wolfe and Cote. This was the measurement of immune response by the production of natural agglutinins to heterologous erythrocytes and implantation of autologous bursa cells in restoration attempts. The effect of bursectomy on natural agglutinin production is directly related to the age of the operation (Mueller, Wolfe and Cote, 1964) and the ontogeny of natural agglutinin production in chickens has been described (Wolfe, Carrol and Cote, 1962; Seto and Henderson, 1968). This implies that the relation of the bursa to natural agglutinin production is restricted to a very early period in life.

Chickens produce natural agglutinins to a variety of heterologous erythrocytes. Antibodies to rat, rabbit and human A, O and B cells have been demonstrated. Their production is attributed to bacterial antigens encountered early in life (Springer, Horton and Forbes, 1959); some of the antibodies to E. coli and other bacteria cross react with

erythrocytes.

I attempted to study the effects of bursectomy (Ⱥ) and immediate replacement of whole autologous bursa (Ⱥ + B) on the production of natural agglutinins to rabbit erythrocytes. The Ⱥ operation was the same described by Sheridan (1967). In the Ⱥ + B operation, the bursa was surgically removed, rinsed in Hanks Balanced Salt solution containing penicillin and streptomycin as described previously for tissue culture, blotted slightly on sterile tissue and simply replaced in situ with blunt forceps. Attempts were made to maintain some degree of sterility in the actual replacement. Operations were performed within three hours of hatching. Wounds were washed once with penicillin-streptomycin solution (100,000 units and 100 ug./ml.), some of which remained within the peritoneum.

During the experiments Hyline Poultry Farm or commercial White Leghorn (De Zeeuws, Edmonton) fertile eggs were hatched in a Jamesway incubator, separated into experimental groups, subjected to treatment and raised together in a battery brooder. All birds were given the antibiotic Tylosin in the drinking water (2 gm./ gall.) the first three days post hatch. Throughout the experiments all birds were raised in groups of six per cage in the same room.

In all experiments natural agglutinins to rabbit erythrocytes were measured as follows. Serum was obtained from 1-2 ml. of brachial vein blood by clotting at room temperature for 1 hour and then at 2-4 degrees C. for 4-6

hours. The microtitre agglutination plate (Cooke Engineering Co., Virginia) technique was used. Antigen was a 2% suspension of washed rabbit erythrocytes diluted in Alsever's solution (Kabat and Mayer, 1961). The serum diluent was 0.88% saline. Antibody was diluted by doubling dilutions from well to well with microtitre delivery loops starting with undiluted serum in the first well. Titres were expressed as $\log_2$ dilution.

RESULTS

Three experiments were carried out. The experimental design consisted of three groups; sham operated controls, bursectomy and replacement. Two experiments were performed with Hyline stock, one with locally obtained commercial stock. The total numbers of the experimental groups surviving for the duration of these experiments were; control, 20; bursectomy, 18 and replacement, 22. These experiments were intended primarily to test the effect of bursectomy and bursa replacement on antibody production and, secondly, to test the hypothesis that bursa replacement would reduce the mortality attributed to bursectomy. During the four months of each experiment there was an overall increase in antibody production but there was considerable variation within and between experiments. To overcome this, the data were analyzed in terms of the mean of all operated individuals in comparison to the control group mean. This type of analysis is presented in Figure 45 for antibody production

and in Figure 46 for the proportion of antibody producing individuals in each operated group.

Antibody is not detectable with the microtitre system until 4-6 weeks of age. Antibody titres increase gradually to a plateau level around 12 weeks of age. That is, serum natural agglutinins increase very rapidly from about 6-12 weeks of age and less rapidly thereon. In these experiments antibody titres were measured at two week intervals from 6 or 7 to 16 weeks of age. In Figure 45 only those birds producing detectable antibody are considered.

At 7 weeks of age bursectomized individual titres were 1.2 Log_2 units lower than control titres and bursectomized-replaced individual titres almost 3 Log_2 units lower. Only 45% of the bursectomized individuals produced antibody in comparison to 100% of the control group and 70% of the bursectomized-replaced individuals (Figure 46). During the following 5 weeks, titres of bursectomized individuals increased gradually to within 0.8 Log_2 of control levels, and the proportion of antibody producing individuals increased to 66% of the control proportion. In contrast, titres of bursectomy-replacement birds, in the same time interval, increased much more rapidly to 1.6 Log_2 above control titres. The proportion of antibody producers increased 10% to 80% of control. In both groups the increase in proportion of antibody producing individuals is linear

over the duration of the experiments. Thus, at 16 weeks of age all of the bursectomy-replacement individuals produced antibody and 80% of the bursectomized individuals produced antibody. From 12 to 16 weeks the higher than control titres of bursectomized-replaced individuals rapidly decreased to meet control levels and the titres of bursectomized individuals maintained the slow rate of increase to meet control levels.

Several effects of bursectomy and bursectomy-replacement became apparent from these data. The immediate effect of bursectomy is to inhibit antibody production in some individuals and to delay the attainment of high titres in other producing individuals. Some of the inhibited individuals recover subsequently, others do not. Figure 47 shows titre frequency distributions at 8 and 16 weeks, illustrating this point. All individuals which do produce antibody initially, at whatever level, eventually produce antibody at nearly the same level as controls.

The effect of replacing the bursa is more striking than the effect of bursectomy. The proportion of individuals which produce little or no antibody is half the proportion in bursectomized individuals. Thus the effect of replacement is detectable six or seven weeks after the operation. However, the amount of antibody each producing individual demonstrates is only one half of that produced by bursectomized individuals and one eighth of that pro-

duced by unoperated controls. Thus, with bursa replacement, the early benefit of more antibody producing individuals is countered by lower individual production. The second and major effect of bursa replacement is an increase in the rate of individual antibody production such that individual titres exceed unoperated control titres by one to two $\log_2$ units. The increase is preceded by a delay in antibody production and is followed by a decline to control levels at 16 weeks of age.

Survival, the second aspect of these experiments, was analyzed in only one of the three experiments; the one employing commercial White Leghorn stock. This was because the Hyline stock simply did not suffer sufficient mortality. This is attributed to the difference in hardiness between Hyline and other commercial stocks (G. R. J. Law, Hyline Poultry Farms, personal communication, 1969). The mortality for this experiment is shown in Figure 48. The raw numbers of the survival data are as follows: 27 of 31 controls, 17 of 22 replaced birds and 10 of 23 bursectomized birds survived to 17 weeks.

The mean survival times of the three groups were, control 109.3 days, replacement 99.6 days and bursectomy 79.4 days. An F test resolved significant differences of means between bursectomy and either of the two other groups. Replacement of the bursa conferred a significant increase in survival in comparison to bursectomy.

In order to relate the presence or absence of the bursa to natural agglutinin titre all the birds of the preceding experiments were killed at 20 weeks of age and the presence or absence of the bursa determined by the histological methods described. This analysis is shown in Table III. The first part of the table refers to bursectomized and replaced birds which had produced consistently high or low titres. Birds which failed to produce natural agglutinins were not included. Birds with a bursa at 20 weeks tend to have approximately double the natural agglutinin production of birds without a bursa.

The second part of the table deals with the presence or absence of the bursa at 20 weeks of age in fourteen birds of each original operated group and shows no indication that the original operation had any real effect on the proportion of birds maintaining a bursa until 20 weeks of age. This meant that I should follow the immediate persistance of a replaced bursa. Bursectomy-replacement operations were performed on a group of 13 newly hatched chicks as described. Two weeks later I could find implants in only 8 of the 13 (61%). There was no evidence of implants in 5 and there were no detectable bursa remnants at the original site in any of the 13. The recovered implants had not grown appreciably although they were attached to the posterior body wall above the cloaca and were vascular.

TABLE III

Autopsy data from replacement experiments, relation of presence or absence of the bursa at 20 weeks to natural agglutinin titre and to the original operation performed.

	20 week autopsy	20 week Mean titre ± S. E.
Bursa present	13	5.9 ± .53
Bursa absent	14	3.2 ± .73

	20 week autopsy	Original Operation Bursectomy	Replacement
Bursa present	9	4	5
Bursa absent	19	10	9

ADULT BURSECTOMY

In one of the replacement experiments previously described, there was an additional experimental group discarded after the start of the experiment. This was a group of birds given an additional bursa with no interference to their own. These birds were in no way different from control birds with respect to antibody production or survival and, for this reason were not incorporated into experimental results.

This group however, did afford an opportunity to test the implied restriction to the early period of life of the relationship between the bursa and natural agglutinin production. Accordingly, at 14 weeks of age, seven individuals were subjected to surgical bursectomy, the remaining three to sham operations. There was no detectable effect on natural agglutinin production over the following six weeks in any of the birds. But, most of the bursectomized birds appeared unhealthy: comb and wattle color was pale and feathers had a wet, straggly appearance. I took blood and peritoneal fluid smears for Giemsa staining anticipating evidence of bacterial infection. There was no such evidence, but the blood smears suggested large differences in total leucocyte counts in bursectomized and sham operated birds. Hemacytometer counts of total peripheral leucocytes did demonstrate such differences (Table IV). This trial experiment provided the basis for the experiments which follow.

TABLE IV

Peripheral leucocyte counts at 19 weeks of age in birds bursectomized or sham operated at 14 weeks of age. (These birds originally were given an additional bursa at hatching with no demonstrable effect on antibody production) An external control (sham operation at hatching) is included.

Operation	Number of birds	Leucocytes/cu.mm. Whole blood ± S. E.
Bx 14 weeks	7	65,000 ± 7,000
Sham 14 weeks	3	38,000 ± 5,000
Sham 1 day	5	36,000 ± 4,500

MATERIALS AND METHODS

An operative technique was devised for surgical bursectomy at 14 weeks of age when lymphoid regression is under way, but the bursa is still relatively accessible. Birds were anaesthetized with sodium pentothal (60 mg/kg body weight). Cloacal and abdominal areas were plucked and washed with 70% ethanol. A skin incision was made from the sternum to the ventral aspect of the right side of the vent. Muscle and peritoneum were separated by blunt dissection. The bursa was located and dissected free from the posterior body wall dorsal to the cloaca. The bursa was lifted free with retractors and the stalk cut close to the cloaca. No sutures were taken in the cloacal stalk. Incisions in peritoneum, muscle and skin were closed with 4-0 and 5-0 chromic gut sutures. Penicillin (30,000 units) and streptomycin (50 mg) were given intramuscularly. Sham operations were identical with the exception of cutting the bursa stalk. Two experiments were set up. The first consisted of a bursectomized and a sham group of four females and one male each. The second consisted of an unoperated control (no antibiotic treatment) group of four females, a bursectomized group of five males and five females, and a sham group of five males and five females.

All birds were maintained in adjacent cages under the same conditions. At the same time and day each week or each two weeks the birds were weighed and blood

and serum samples taken. Leucocyte counts and natural agglutinin titres to rabbit erythrocytes were measured through the fifth post operative week (19 weeks of age). A series of blood samples were taken over 24 hours from two bursectomized and two sham operated birds to measure diurnal leucocyte count variation. Total leucocyte counts and weight data from both experiments were pooled according to sex in order to reduce some of the variation.

RESULTS

There were no post operative deaths in the first experiment. One bursectomized female died three weeks post operation in the second experiment. Pooled female weights and natural agglutinin titres are shown in Figure 49. Mean weights of bursectomized birds were initially and consistently 40-50 gm. less than sham operated birds. At the termination of the experiment (20 weeks of age) all female birds were in a weight range of 1050 to 1400 gm. There was no indication of a weight loss attributable to bursectomy. Natural agglutinin titres of bursectomized birds tended to be lower than sham titres in the second, third and fourth weeks. The differences were not significant.

The means and standard errors of total leucocytes counts /cu. mm. are shown in Figures 50 and 51 for females and males respectively. In females, two extremes are apparent; extremes of high and low counts and, extremes of variation in leucocyte count from week to week. For example,

unoperated birds tended to have the highest means, sham operated birds the lowest means and bursectomized birds means fluctuated between the two extremes from week to week. To illustrate, the five week average of week to week fluctuation of the mean leucocyte counts for bursectomized females was 14,220 and for sham operated females, 5,024. The comparative variation in leucocyte counts at any one week is similar to the weekly fluctuations. Means of the standard errors were; bursectomized females - 7,214, sham operated females - 5,105, unoperated females - 3,706.

In male birds (Figure 51), the same tendencies are apparent. For example, the mean week to week fluctuation of leucocyte count means from the second to fourth weeks for bursectomized birds was 14,600 and for sham operated birds was 3,455. The mean standard errors were respectively 5,750 and 5,308.

The peripheral leucocyte counts of all individuals tended to increase or decrease from week to week together. A similar cyclic diurnal increase and decrease was observed in the four birds tested (Figure 52) but the maximum 24 hour variation was not more than 10% of the maximum individual week to week variation.

DIFFERENTIAL VARIATION

During the experiments several differential counts of Giemsa stained blood smears were made. Granulocytes were between 10 and 14 per cent of the total cells counted. No

bacteria were ever observed in any of the smears.

EFFECT OF ADULT BURSECTOMY ON SPECIFIC ANTIBODY PRODUCTION

Since there was an indication that bursectomized adults were partially deficient in natural agglutinin production, it was logical to test their ability to respond to specific challenge. This was done in the fifth post operative week of the first experiment. Five females and one male of the bursectomized and the sham operated groups were injected intravenously with one ml. of a 50 per cent suspension in Alsever's solution of washed chicken erythrocytes of another genotype. The recipients were B2B14 and the cells B13B13. Normally, up to three injections over three weeks are required to elicit high serum antibody titres in this system (David et al., 1966). I anticipated a weak response which might be sensitive to impairment by bursectomy. Serum titres were measured 5 and 7 days after challenge. No difference between bursectomy and sham operation was detected (Table V).

INFLUENCE OF ADULT BURSECTOMY ON OTHER LYMPHOID ORGANS

The extreme variations I observed in leucocyte counts in bursectomized birds suggested there might be a detectable effect of the operation in the spleen or the thymus. Another reason for examination of the lymphoid organs was the possibility of bursa regrowth. Accordingly, at the end of the experiment, birds were weighed, killed, examined for residual bursa tissue, and the spleen,

TABLE V

Antibody production in response to injection of B13B13 chicken erythrocytes into B2B14 birds.

Operation	Titres and Mean Titres Days after injection 5	7
Bursectomy	3,4,0,1,1,1 1.6	6,6,6,6,7,4 5.8
Sham	0,1,2,2,3,3 1.8	3,4,4,5,5,6,8 5.0

residual bursa, if any, and four lobes of the thymus were taken from a number of individuals in each group, weighed and fixed for histological examination.

All of the shams possessed a typical twenty week regressing bursa. Some bursectomized birds had residual bursa tissue but it was always encysted and atypical. In detail, the average weight of residual bursa in three of nine bursectomized females was 0.374 gm. In six sham operated females the average weight of residual bursa was 1.79 gm. and in the four unoperated females, 0.944 gm. In three of four bursectomized males the average bursa weight was 0.252 gm. and in three of four sham operated males, 1.112 gm. This indicates that bursectomy at fourteen weeks was often incomplete, but that there was little or no regrowth of the bursa.

Spleen weights expressed as per cent of body weight tended to be different but there was not sufficient individuals to detect significance. Bursectomized females and males had heavier spleens than sham or unoperated controls and males had heavier spleens than females (Table VI).

Bursectomized females tended to have heavier thymus lobes than sham and unoperated controls but in males, the tendency was opposite.

In summary, bursectomy at fourteen weeks of age effects a disturbance of peripheral leucocyte kinetics in both males and females; it tends to effect a splenic en-

largement in both males and females and it tends to effect a thymic enlargement in females only.

If there is a thymic enlargement in females, the question arises; is it due to enlargement of the cortex, the medulla or both. Histological preparations were made of several thymus lobes from each of five bursectomized and five sham operated females. Measurements of the total thickness of cortex and medulla across the widest area of each thymus lobe were made in terms of ocular micrometer units. The mean values were obtained from at least 10 different sections for each individual. Every bursectomized bird demonstrated measurable cortex tissue and the mean ratio of cortex-medulla was 0.77. Two of five sham operated females had no measurable thymic cortex and the mean ratio was 0.10. These values of cortex and medulla thickness were plotted and the regression lines calculated (Figure 53). The two lines appear to be very different and a t test of slope was significant (p .05). Thymic enlargement in bursectomized females is evident in cortical thickness which is related to the absolute size of the medulla.

TABLE VI

Spleen and thymus weights in relation to body weight at 20 weeks in birds bursectomized, sham operated or unoperated at 14 weeks of age.

Operation	(N)	Spleen weight per cent body weight$X10^{-5}$	4 lobes thymus per cent body weight$X10^{-5}$
Females			
Bursectomy	6	203.1 ± 14.1*	88.5 ± 18.8
Sham	8	186.1 ± 10.1	59.3 ± 18.2
Unoperated	4	175.0	52.2
Males			
Bursectomy	4	242	114.9
Sham	4	190	129.8

* - Standard error calculated. No differences were significant.

DISCUSSION

The basic purpose of this study is a consideration of the persistence and function of the epithelium of the bursa of Fabricius of the chicken. The approaches I chose were simple. First, I examined the physical characteristics of the epithelium of the organ in very old birds, in young adult birds and in very young birds. Second, I examined the immunological consequences of removal and replacement of the organ in very young birds and of removal of the organ in young adults. Three principal questions were asked; how long and in how many birds does the epithelium of the bursa persist, does it function particularly in the adult, if so, how?

In answer to the first question, I found persisting epithelium in 77 per cent of White Leghorns and 79 per cent of the other breeds examined in the age range of 12 to 34 months. The implication is obvious; bursa epithelium is present in three of four adult birds. As for how long the epithelium persists, the most appropriate statement is that the presence or absence of the epithelial component of the bursa cannot be predicted for any mature individual. Bursa epithelium was found in birds as old as four years and was not found in birds as young as six months. This

is consistent with Jolly's original study in 1915 but my finding of 78 per cent epithelial persistence in adults, is not. However, an analysis of the distribution of birds with and without persisting epithelium according to age suggested a possible relation between age and epithelial persistance and survival. The greatest incidence of birds without a bursa is from 6 to 10 months. This corresponds closely to the period of the most severe mortality. The obvious suggestion is that those birds in which the bursa disappears from 6 to 10 months tend to die in the same period. This interpretation confers a considerable survival value on epithelial persistance. An alternative interpretation is that the loss of the epithelium is an indication but not a cause of impending mortality.

The question of the identity of persisting epithelium with younger enlarging bursa epithelium had to be satisfied before immunological questions could be asked. This was accomplished through morphological, histochemical and functional studies of growing, regressing and regressed bursa epithelium. The rapid changes due to growth and regression of the lymphoid tissue of the bursa are only partially reflected in the epithelium during the five or six months the lymphoid cycle occupies. Between three and five months, the epithelium becomes less complex in organization and a specific cell population disappears. The disappearance is evident in the disappearance of a morphological class

of nuclei and in the reduction of the epithelium to a simpler organization. The nuclear class in question is ellipsoid, hyperchromatic and has one or two nucleoli (Figure 22). This fits a goblet cell population described in very young epithelium (Ackerman and Knouff, 1959). Goblet cells, however, are as numerous in persisting epithelium as in young, lymphoid bursa epithelium. These epithelial changes are interpreted to mean that at three months there is a population of basal cells which differentiate into elongate columnar cells. At six to seven months, a considerable proportion of the columnar cells are no longer present but the population of basal and presumable primitive mitotic cells remains.

Histochemical comparisons revealed no difference in bursa epithelium throughout lymphoid regression.

The appearance during lymphoid regression of a new and distinct epithelium in the bursa, designated as cyst epithelium, is a result of lymphoid follicle degeneration. This epithelium becomes apparent as the medulla of the follicle degenerates, and is the remaining undifferentiated epithelial cell layer which separates cortex and medulla (Ackerman and Knouff, 1959). Cyst epithelium may persist as such in order to contain the products of follicle degeneration. Similar structures have been described in atrophic mammalian thymus (Glucksmann and Cherry, 1968). Histochemically and morphologically, cyst epithelium is dis-

tinct from persisting bursa epithelium; the remaining part of this study pertains only to persisting epithelium.

The histochemical studies demonstrated several identifying characteristics of bursa epithelium of any age. These are: alkaline phosphatase activity adjacent to the basal cells, acid phosphatase activity in the cytoplasm adjacent to the lumen and considerable RNA content of the cytoplasm. Bursa alkaline phosphatase activity has been related to lymphopoiesis (Ackerman and Knouff, 1963). The persistence of this enzyme activity long after lymphopoiesis has ceased at this site would suggest that the relationship of the enzyme activity to lymphopoiesis only be incidental.

Acid phosphatase in developing bursa epithelium has been associated with the formation and disposal of debris (Chen, 1968). The presence of potential acid phosphatase containing structures (Figure 23) and the demonstration of acid phosphatase activity in this area in all ages of epithelium could indicate a continuation of this type of activity.

RNA content of epithelial cytoplasm is an indication of RNA synthesis. This was substantiated by tritiated uridine uptake experiments. These experiments demonstrated a possible epithelial absorption of material injected into the lumen of the bursa and transport through the epithelium to the follicular cortex or tunica propria in non lymphoid bursas. Conversely, intravenously administered labelled

uridine appeared in epithelial cytoplasm. All together, the results obtained indicate a continuing spectrum of synthetic and potentially absorptive activities in bursa epithelium which are probably independent of the lymphoid activities of the bursa.

Other aspects of the functional studies support this idea. Within the limits of the techniques employed, bursa innervation is not modified during lymphoid regression.

A comparison of mitotic activity in enlarging and persisting epithelium demonstrated a shift in location of DNA synthesizing cells. In lymphoid bursas, the mitotic cells are restricted to epithelium between follicles. There is less extensive incorporation in follicle tissues. Mitotic cells in non-lymphoid bursas are not so strictly located in the epithelium. Many cell clusters are in the stroma or tunica propria close to the base of the epithelium. Although the location of DNA synthesis shifts slightly, there is no apparent loss of this activity during regression.

Thymidine incorporation in cells in the degenerating lymphoid follicles occasionally present at 7 months of age may represent phagocytic activity of cells participating in follicle destruction.

Some conclusions may be drawn from the studies just discussed:

Some epithelial cell population(s) disappear during regression of bursa lymphoid tissue.

Other epithelial cell population(s) persist. These population(s) are part of bursa epithelium from the early stages of organ growth and are present throughout the life of the organ.

Such cells are not necessarily directly functionally related to lymphopoiesis in the bursa but may be indirectly related to some other immune function(s).

Before attempting to define any function of persisting epithelium it was necessary to attempt a demonstration of the function of pre-regression epithelium. If the relation of the neonatal bursa to development of antibody producing cells is definitive, immune activities lost by bursectomy should be partially restored if the organ can be replaced. This has been previously attempted but the results have been somewhat ambiguous. Immune unresponsiveness due to surgical or chemical bursectomy has been partially overcome by repeated immunizations (Jankovic and Isakovic, 1966), by implantation of bursas (Isakovic and Jankovic, 1964) and by implantation within millipore filter chambers (St. Pierre and Ackerman, 1965). There is reason to believe that some of these positive results may be due to the adjuvant action of contamination of transplants and chambers by bacteria (Dent and Peterson, 1967).

I attempted to avoid such problems in restoration experiments by implanting autologous bursa within minutes of extirpation and by measuring immune response in terms of natural agglutinin production thus avoiding samp-

ling stimulation. This type of experiment was suggested by Mueller, Wolfe and Cote (1964). The results I obtained permit the following generalizations:

(1) The effect of bursectomy is transitory. Most birds tend to recover within 4 or 5 months.

(2) The effect of bursectomy is to delay natural agglutinin production in most birds and to suppress it in others. In a few individuals the operation caused a short term stimulation of natural agglutinin titres with no effect beyond that. This may reflect incomplete bursectomy or individual differences in the time course of bursa development.

(3) Replacement of the bursa restores the antibody producing capacity in some of the birds in which this capacity is lost. Replacement, at first, tends to delay antibody production (in terms of individual titres) for a short time and then to stimulate antibody production for some six weeks. By twenty weeks the stimulation is not apparent and all groups tend to have approximately the same titre range.

(4) Bursectomy causes severe mortality. Replacement of the bursa significantly restores survival.

These findings relate antibody production and survival to the presence of the bursa but not necessarily to the presence of bursa epithelium. Autopsy showed a relation between the presence of persisting epithelium and a higher

antibody titre, but the same data and the two week autopsy clearly showed that implanted bursas did not persist *in situ* until the end of the experiments and, even if implanted successfully, failed to develop further. Thus, the restorative action of the bursa is real, but any relation of neonatal epithelium to the restorative action is by implication only.

Bursectomy, as little as one month after hatching, does not appreciably affect antibody producing capacity (Mueller, Wolfe and Cote, 1964). This implies no functional relation of the bursa or its epithelium to antibody production at this time. If there is a relation of either to some other immune function, extirpation of the bursa later than one month should effect some immune function other than antibody production. This was the reasoning which prompted bursectomy at fourteen weeks. The first attempt at fourteen week bursectomy did not reveal any impairment of natural agglutinin production. It did indicate a considerable increase in peripheral leucocytes in blood smears. (The smears were taken for bacterial staining because the birds appeared unhealthy, but there was no evidence of bacterial infection). Leucocyte counts of whole peripheral blood were elevated in comparison to sham operated birds or birds bursectomized at hatching. A repeated series of similar operations revealed a slight decrease in natural agglutinin production in the third week after the operation but there was no impairment of antibody production in response to specific challenge.

One of fifteen birds died after the operation. There was no effect of the operation on body weight or survival within the six weeks after the operation.

A marked effect of the operation was detected in peripheral leucocyte counts in the six weeks following the operation. This was a considerable increase in the amplitude of variation of total leucocyte counts in comparison to unoperated and sham operated controls. The whole population of birds in the experiments demonstrated a remarkable uniformity in the tendency of total leucocyte counts to increase or decrease from week to week. This cyclic fluctuation was exaggerated in bursectomized birds.

Another effect of the operation was a decrease of total leucocyte counts in comparison to unoperated controls. Bursectomized females tended to have consistently lower counts than unoperated controls and sham operated females tended to have lower counts than bursectomized females. Bursectomized males tended to have lower total leucocyte counts than sham operated males. Sheridan (1967) reported a similar effect of depression of leucocyte counts due to bursectomy at hatching in birds seven to twelve weeks old. The extreme difference between unoperated and operated female leucocyte counts is puzzling. An explanation may be partially due to a treatment difference. Unoperated controls did not receive antibiotic treatment at the beginning of the experiment but all other birds did.

There is no reason to believe that this flock was initially free of bacterial or other infection. The antibiotic treatment of sham operated birds may have reduced their bacterial population in comparison to that of unoperated controls and thus effected a lesser immune stimulation.

An explanation of the exaggerated variation of leucocyte counts in bursectomized birds required an examination of other lymphoid tissues. This demonstrated a clear tendency towards increased spleen weight in both sexes and towards increased thymus weights in females only. Thymic enlargement was shown to be a real effect, evident in the cortex relative to the medulla in females. These results (Table VI) could be taken to indicate a sex difference in response to bursectomy for which there is precedent in terms of decreased graft versus host capability in bursectomized males (Sheridan, 1967) but the numbers of birds are not sufficient to permit this interpretation in my data.

Statistical treatments of the peripheral total leucocyte counts were not applied. This is because leucocyte counts of chickens are extremely variable. Lucas and Jamroz (1961) estimate that one hundred thirty chickens must be studied to obtain a lymphocyte mean for a population with 95 per cent confidence that it falls within limits of 10 per cent. This simply was not possible in

this study. The results of adult bursectomy I obtained can therefore only be interpreted in terms of the tendencies which were apparent.

At fourteen weeks of age, lymphoid regression is in progress. Lymphoid follicles are approximately half the size they were at ten weeks of age. Removal of the bursa at this time really means the removal of both epithelial and lymphoid components of the bursa some two weeks after the maximum organ size has been reached. Maximum organ size more closely reflects maximum lymphoid development than it does maximum epithelial development. This study has established that the epithelial component will persist for a long time after fourteen weeks and that the lymphoid component will not. Thus, the implication is that the effects of adult bursectomy are more related to the epithelial component than to the regressing lymphoid component. The fact that neonatal bursectomy was clearly related to the immune function of antibody production but that this relation was not demonstrated by bursectomy at fourteen weeks lends support to the implication.

Clearly, this study has provided evidence demonstrating that the immunological importance of the bursa of Fabricius extends well into adult life. The nature of the evidence implicates the persisting epithelium in a relationship with spleen and thymus in maintaining homeostasis of the lymphoid system.

SUMMARY

(1) A histological survey of the presence or absence of the bursa of Fabricius showed persistence of bursa epithelium in 77% of adult White Leghorn and 79% of White Rock, Black Rock and Barred Rock chickens examined. These data were interpreted to suggest that bursa epithelium persists throughout life.

(2) Light and electron microscopic studies indicated that a population(s) of epithelial cells disappears during lymphoid regression and epithelial cell population(s) which persist, are identical with cell population(s) present from early stages of bursa growth.

(3) Histochemical studies confirmed these findings.

(4) The appearance of a different epithelium during lymphoid regression is described.

(5) The innervation of the bursa did not appear to change during lymphoid regression.

(6) Bursa epithelium of all ages is notable for RNA content, acid phosphatase content and alkaline phosphatase activity associated with the basal area.

(7) Epithelium of growing and regressing bursas was shown to synthesize RNA as measured by H^3-uridine uptake.

(8) The location of DNA synthesis as indicated by H^3-thymidine

uptake was shown to shift to some extent from epithelial cells in lymphoid bursas to subadjacent cells in the tunica propria in non lymphoid bursas.

(9) Neonatal surgical bursectomy inhibited the production of natural agglutinins to rabbit erythrocytes. Natural recovery returned agglutinin titres to control levels in four or five months.

(10) Immediate replacement of excised bursas accelerated normal recovery after a short delay.

(11) Neonatal surgical bursectomy severely increased mortality. Replacement of the bursa ameliorated mortality due to bursectomy.

(12) Surgical bursectomy in adults produced entirely different effects than the neonatal operation. Bursectomy at 14 weeks of age significantly increased magnitude of peripheral leucocyte count variation for five weeks and effected a clear tendency towards an increase of thymus weight and spleen weight in females and an increase of spleen weight in males.

(13) The suggested explanation is that the operation removed a homeostatic restraint on the lymphoid system.

(14) A further implication is that the persisting epithelium is involved in the proposed homeostatic restraint.

LITERATURE CITED

Ackerman, G. A. and R. A. Knouff. 1959. Lymphocytopoiesis in the bursa of Fabricius. Am. J. of Anatomy. 104: 163.

Ackerman, G. A. and R. A. Knouff. 1963. Testosterone suppression of mesenchymal alkaline phosphatase activity and lympho epithelial nodule formation in the bursa of Fabricius of the embryonic chick. Anat. Rec. 146: 23.

Arthur, J. P. and W. B. Shelley. 1959. The technology of IN VITRO staining of nerves in human skin with thiazin dyes. J. of Investigative Dermatology 33: 121.

Barka, T. and P. J. Anderson. 1965. Histochemistry. Theory, Practice and Bibliography. Harper and Row. New York.

Bertalanffy, F. D. 1962. Evaluation of the Acridine-Orange fluorescence method for cytodiagnosis of cancer. Ann. N. Y. Acad. of Science. 93: 715.

Chen, L. T. 1968. Primordia of Lymphoid Organs of the Chicken. Thesis. University of Alberta.

Cheville, N. F. 1967. Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of the chicken. Am. J. of Pathology. 51: 527.

Cooper, M. D., W. D. Cain, P. J. Van Alten and R. A. Good. 1969. Development and Function of the Immunoglobulin Producing System. 1. Effect of bursectomy at different stages of development on germinal centers, plasma cells, immunoglobulins and antibody production. Int. Arcy. of Allergy and Appl. Immunology. 35: 242.

Corrodi, H., G. Jonsson, T. Malmfors. 1966. Factors affecting the quality and intensity of the fluorescence in the histochemical method of demonstration of catecholamines. Acta. Histochem. 25: 367.

Culling, C. F. A. 1963. Handbook of Histopathological Techniques. Butterworths, London.

David, L. A., R. F. Ruth, G. R. J. Law. 1966. Specific detection of cellular transplantation antigen by fluorescent antibody. Ann. N. Y. Acad. of Science, 129: 46.

Dent, P. B. S. and R. D. A. Peterson. 1967. Bursa hormone-a non specific adjuvant effect of bursal diffusion chamber implants on antibody production. Fed. Proc. 26: 621.

Ehinger, B., B. Sporrong, U. Stenevi. 1967. Combining the catecholamine fluorescence and methylene blue staining methods for demonstrating nerve fibres. Life Sciences. 6: 1973.

Falck, B. 1962. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. Acta. Physiologica Scand. 56 supp. 197.

Glick, B. 1956. Normal growth of the bursa of Fabricius in chickens. Poultry Science. 35: 843.

Glick, B. 1969. Hematology of chemically bursectomized birds. Avian Diseases. 13: 142.

Glucksmann, A. and C. P. Cherry. 1968. The effect of castration, oestrogens, testosterone and the oestrous cycle on the cortical epithelium of the thymus in male and female rats. J. of Anatomy. 103: 113.

Isakovic, K. and B. D. Jankovic. 1964. Role of the thymus and the bursa of Fabricius in immune reactions in chickens. Int. Arch. Allergy. 24: 296.

Jankovic, B. D. and K. Isakovic. 1966. Antibody production in bursectomized chickens given repeated injections of antigen. Nature. 211: 202.

Jolly, J. 1915. La Bourse De Fabricius et des Organes Lympho-Epitheliaux. Arch. Anat. Micr. 16: 363.

Kabat, E. A. and M. M. Mayer. 1961. Experimental Immunochemistry. C. C. Thomas. Springfield, Ill.

Karnovsky, M. and L. Roots. 1964. A direct thiocholine method for cholinesterases. J. Histochem. Cytochem. 12: 219.

Lucas, A. M. and C. Jamroz. 1961. Atlas of Avian Hematology. U. S. Department of Agriculture. Washington, D. C.

Luft, J. H. 1961. Improvements in Epoxy resin embedding methods. J. of Biophysical and Biochemical Cytology. 9: 409.

Meyer, R. K., M. A. Rao and R. L. Aspinall. 1959. Inhibition of the development of the bursa of Fabricius in the embryo of the common fowl by 19 nor-testosterone. Endocrinology. 64: 890.

Mueller, A. P., H. R. Wolfe and R. K. Meyer. 1960. Precipitin production in chickens. Antibody production in bursectomized chickens and in chickens injected with 19 nor-testosterone on the fifth day of incubation. J. of Immunology. 85: 172.

Mueller, A. P., H. R. Wolfe and W. P. Cote. 1964. Antibody studies in hormonally and surgically bursectomized chickens. In R. A. Good and A. E. Gabrielson. The Thymus in Immunobiology. Harper and Row. N. Y.

Parker, R. C. 1961. Methods in Tissue. Harper and Row. N. Y.

Pearse, A. G. E. 1961. Histochemistry. Theoretical and Applied. J. A. Churchill Ltd. London.

Prescott, D. M. 1964. Autoradiography with liquid emulsion. In Methods in Cell Physiology. Vol. 6. Academic Press, N. Y.

Saint Pierre, R. L., G. A. Ackerman. 1965. Bursa of Fabricius in Chickens: Possible humoral factor. Science. 147: 1307.

Scott, J. E., G. Quintarelli and M. C. Dellova. 1964. The chemical and histochemical properties of Alcian blue. Histochemie 4: 73.

Seto, F. and W. G. Henderson. 1968. Natural and immune hemagglutinin forming capacity of immature chickens. J. of Exp. Zool. 169: 501.

Sheridan, S. 1967. Lymphoid tissue and immune Activities of the chicken: Alteration by bursectomy, orchiectomy and thymectomy. Thesis. University of Wisconsin.

Spicer, S. S. and R. D. Lillie. 1961. Histochemical identification of basic proteins with Biebrich Scarlet at alkaline pH. Stain Technology. 36: 365.

Springer, G. F., R. E. Horton and M. Forbes. 1959. Origin of antihuman blood group B agglutinins in germfree chicks. Ann. N. Y. Acad. of Science. 78: 272.

Stevens, H. and R. L. Mainwaring. 1967. Modified hematoxylin and eosin staining technique for frozen section. Am. J. of Medical Technology. 33: 148.

Szirmai, J. A. 1963. Quantitative approaches in the histochemistry of mucopolysaccharides. J. Histochem. Cytochem. 11: 24.

Warner, N. L. and F. M. Burnet. 1960. The influence of testosterone treatment on the development of the bursa of Fabricius in the chick embryo. Austral. J. of Biol. Science. 14: 580.

Wolfe, H., M. Carroll and W. Cote. 1962. The effect of bursectomy of the chicken on the formation of precipitins and hemagglutinins. Fed. Proc. 21: 22.

APPENDIX A

Table 1 - Explanation of table. Dimensions were measured with a calibrated ocular micrometer and represent the extreme values of several observations. The identification of Alcian Blue positive cells and Periodic-acid-Schiff positive cells was from sections stained by a combined Alcian-blue-PAS method (Culling, 1964). u = micron

TABLE I

	13 month proctodeal epithelium	13 month coprodeal epithelium	10-15 month persisting bursa epithelium	1-3 month bursa epithelium
epithelial thickness	22-30 u	28-45 u	12-20 u	18-30 u
description	columnar	pseudostrat columnar	pseudostrat columnar	pseudostrat columnar
number of cell types	2	3	2	3
nuclear shapes and diamters	oval 6 u round 4 u	cigar 8 u round 6 u	oval 4 u round 4.5 u	cigar 8 u round 4.5 u round 6 u
number of levels of nuclei	2	4	3-4	4-5
goblet cells	no	yes	no	no
PAS positive secretory cells	yes	yes	no	no
Alcian blue positive secretory cells	yes	yes	yes	yes
Alcian blue positive surface coat	yes	yes	yes	yes
epithelial gland crypts	yes	yes	yes	no

Table 2 - Explanation of table. O = ortochromatic stain, + = positive reaction, intensity denoted by number of +'s, ABPAS = combined Alcian blue PAS procedure, AB = Alcian blue positive staining, hyaluronidase = hyaluronidase digestion according to Pearse, 1961, RNAse = ribosenuclease digestion according to Culling, 1964, trypsin = trypsin digestion (Culling, 1964), - = negative reaction.

TABLE 2

Histochemistry of bursa epithelium

TREATMENT	Growing (3 month) epithelium		Persisting (13 month) epithelium	
	surface coat	cells	surface coat	cells
Azure A pH 3.9	0 ++++	0 ++++	0 ++++	0 ++++
hyaluronidase Azure A pH 3.9	0 +++	0 ++	0 +++	0 ++
RNAse Azure A pH 3.9	0 ++	0 ++	0 ++	0 ++
ABPAS	AB +++	AB ++	AB +++	AB ++
hyaluronidase ABPAS	" "	" "	" "	" "
trypsin ABPAS	AB +	-	AB +	-
Acridine orange	++	++	++	++
RNAse Acridine orange	-	-	-	-
Biebrich scarlet pH 6.5	++	+	++	+
pH 8.0	+	-	+	-
pH 10.5	-	-	-	-

APPENDIX B

FIGURES AND EXPLANATION OF FIGURES

Figure I - This figure represents the results of histological examination of 78 White Rock, Black Rock and Barred Rock chickens obtained from the Dept. of Poultry Science, University of Alberta, to ascertain the presence or absence of the bursa.

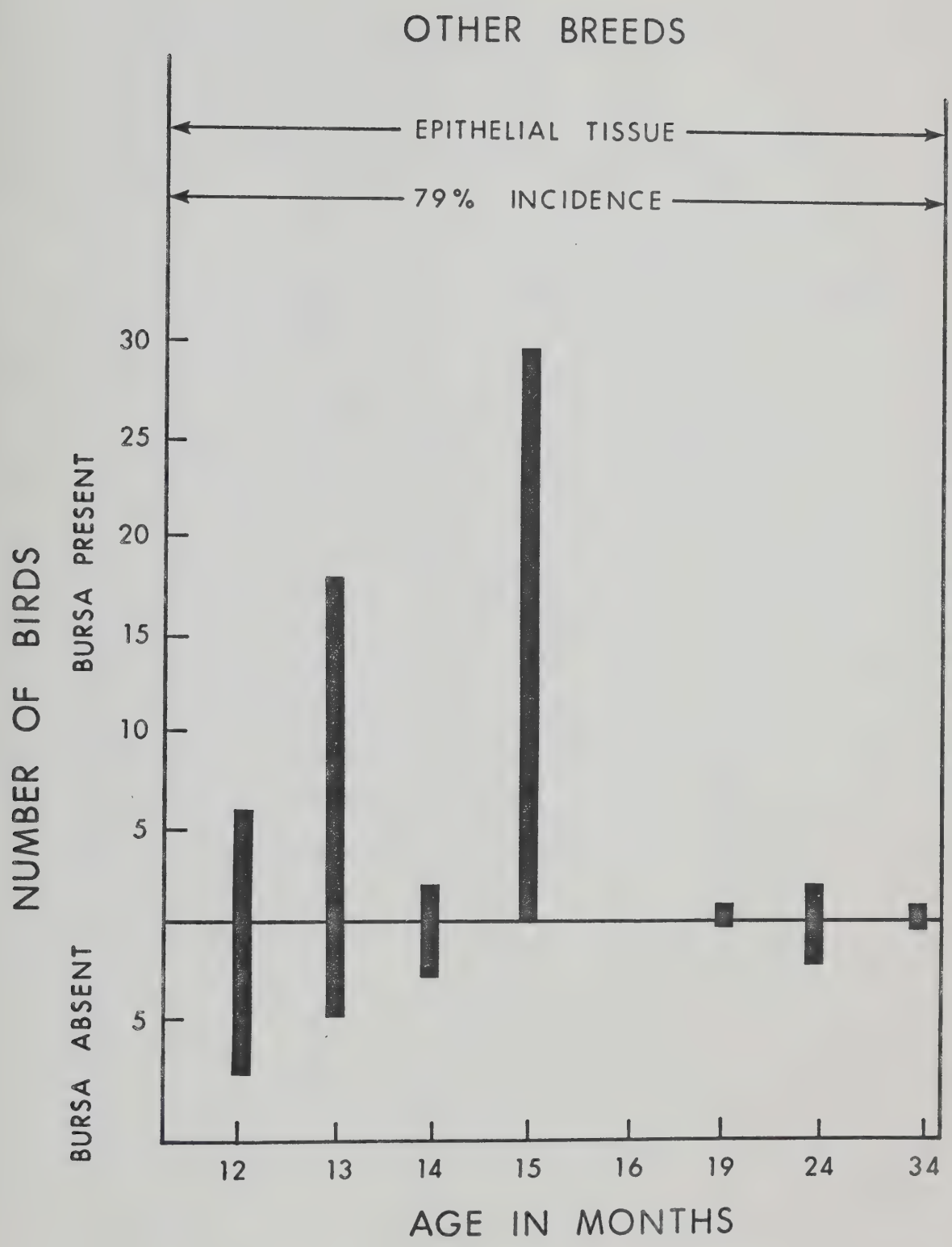

OTHER BREEDS
EPITHELIAL TISSUE
79% INCIDENCE
NUMBER OF BIRDS
BURSA PRESENT
BURSA ABSENT
30
25
20
15
10
5
5
12
13
14
15
16
19
24
34
AGE IN MONTHS

Figure 2 - This figure represents the results of histological examination of 60 Single Comb White Leghorns to ascertain the presence or absence of the bursa.
Note (1). Regression of the bursa in Single Comb White Leghorns begins between 12 and 14 weeks of age when measured by organ weight decrease.
Note (2). Incidence refers to the per cent proportion of birds with demonstrable bursa present of all the birds examined in the indicated age bracket.

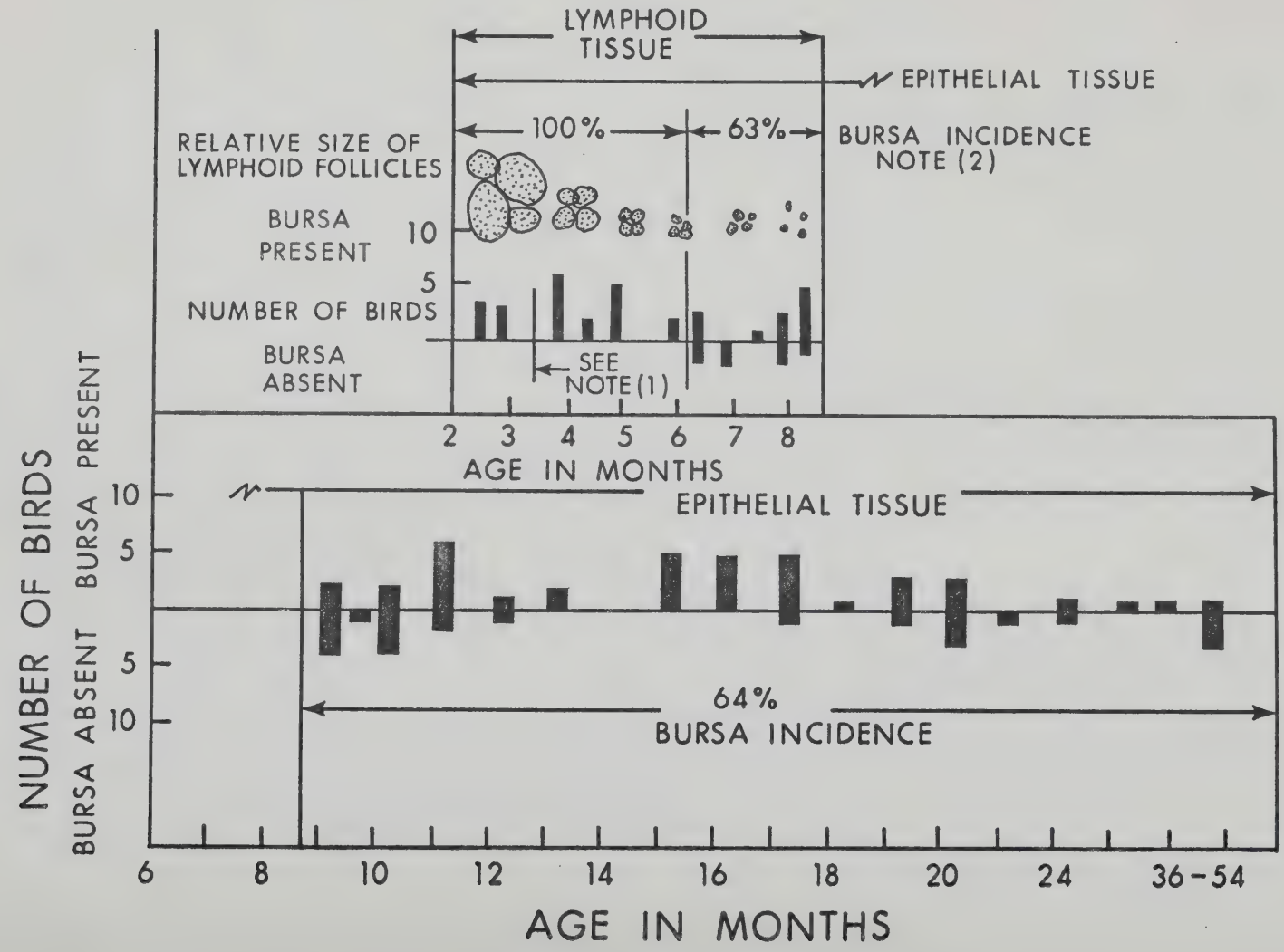

LYMPHOID TISSUE
EPITHELIAL TISSUE
100%
63%
BURSA INCIDENCE NOTE (2)
RELATIVE SIZE OF LYMPHOID FOLLICLES
BURSA PRESENT
10
5
NUMBER OF BIRDS
BURSA ABSENT
SEE NOTE (1)
2 3 4 5 6 7 8
AGE IN MONTHS
NUMBER OF BIRDS
BURSA ABSENT BURSA PRESENT
10
5
5
10
EPITHELIAL TISSUE
64%
BURSA INCIDENCE
6 8 10 12 14 16 18 20 24 36-54
AGE IN MONTHS

Figure III

This figure represents survival data from 28 White Leghorns and the proportion of other White Leghorns over the same age range with and without bursas. Both groups of Leghorns are from the same hatch and were raised under similar conditions. The survival curve was obtained from regression analysis. The bursa proportion line was obtained from the histological analysis of bursa distribution shown in Figure 2.

The survival curve indicates that any statistical comparisons of proportions of bursa positive birds should be based on age ranges of 0-165, 165-275, 275-550 days. A chi square test of the hypothesis that all age ranges reflect the same population distribution of bursa presence or absence was highly significant.

	0 - 165		165-275		275-550	
	O	E	O	E	O	E	Total
+	19	13.528	16	19.936	37	38.448	72
-	0	5.472	12	8.064	17	15.552	29
							101

$$X^2 = \frac{(O-E)^2}{E} = 10.571$$

$$X^2_{.01} \text{ for } = 9.21$$

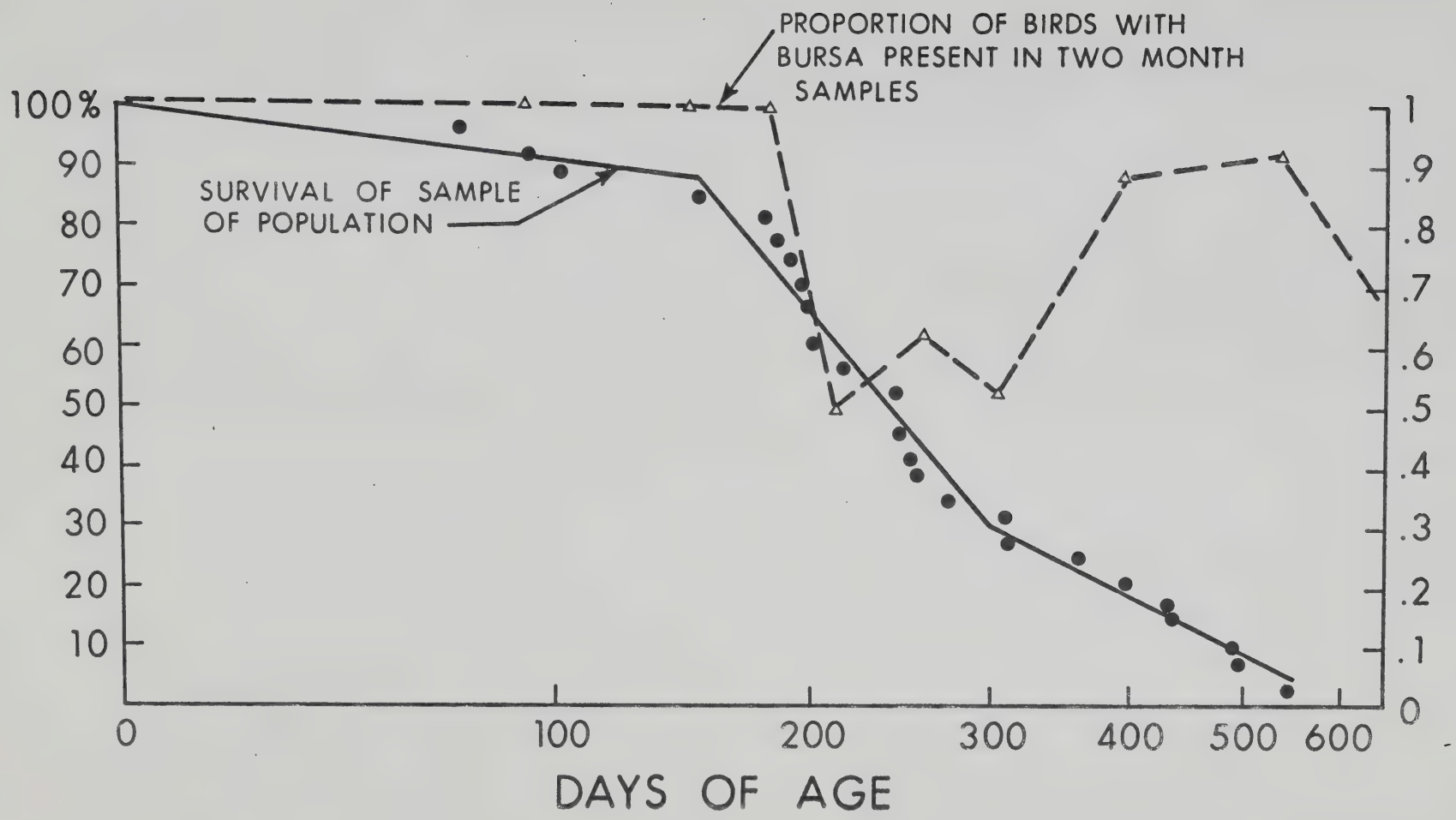
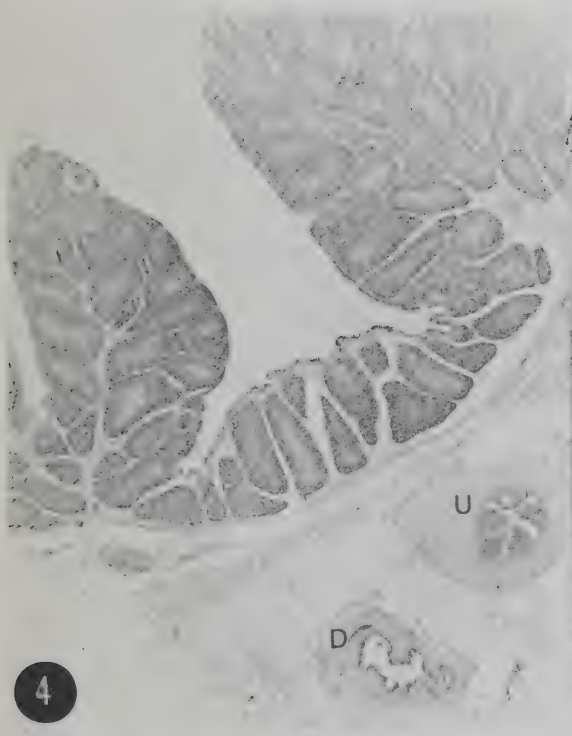

PROPORTION OF BIRDS WITH BURSA PRESENT IN TWO MONTH SAMPLES
SURVIVAL OF SAMPLE OF POPULATION
100%
90
80
70
60
50
40
30
20
10
1
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
0
100
200
300
400
500
600
DAYS OF AGE

Figure 4 - Segment of month old bursa. Hematoxylin eosin.
X 15. U : ureter
D : ductus deferens

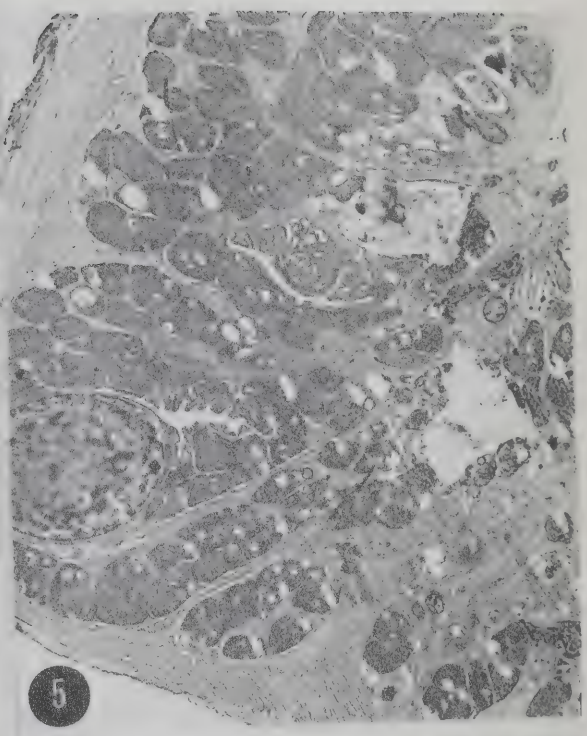
Figure 5 - Segment of 18 week old bursa. Hematoxylin and eosin. X 10. Degeneration of lymphoid follicles is extensive, small and large colloid filled cysts are present in most follicles. C : cyst

Figure 6 - Sagittal section of 5 month old bursa. Hematoxylin and eosin. X 10. Lymphoid regression is almost complete, lymphoid follicles are much reduced in number and size, epithelium is condensed into the stalk region. C : cloacal area L : localization of lymphocyte-like cells.

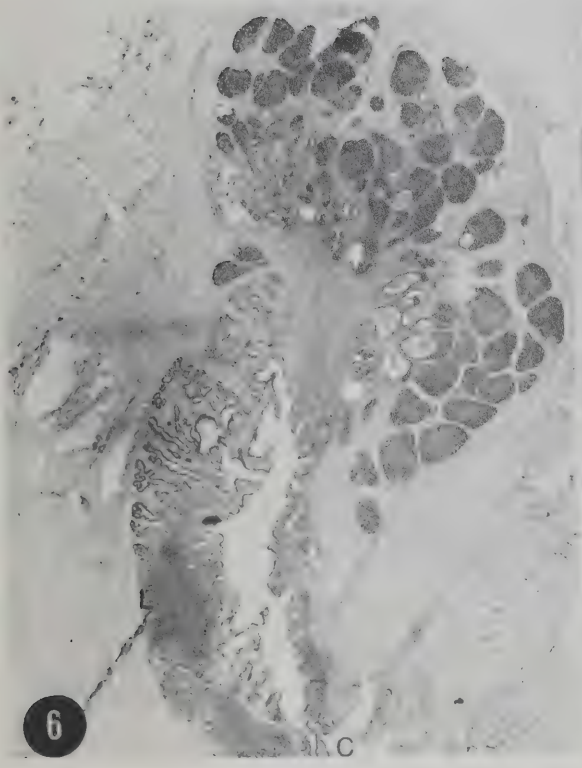
Figure 7 - Sagittal section of 15½ month bursa. Hematoxylin eosin. X 16. Lymphoid re-regression is complete, no follicles are present, and there are only a few areas of lymphocyte like cells.



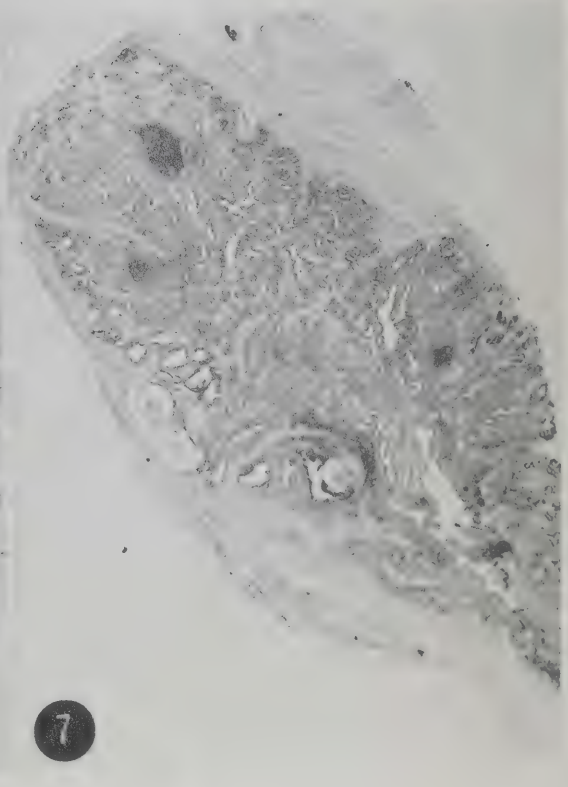
4



5



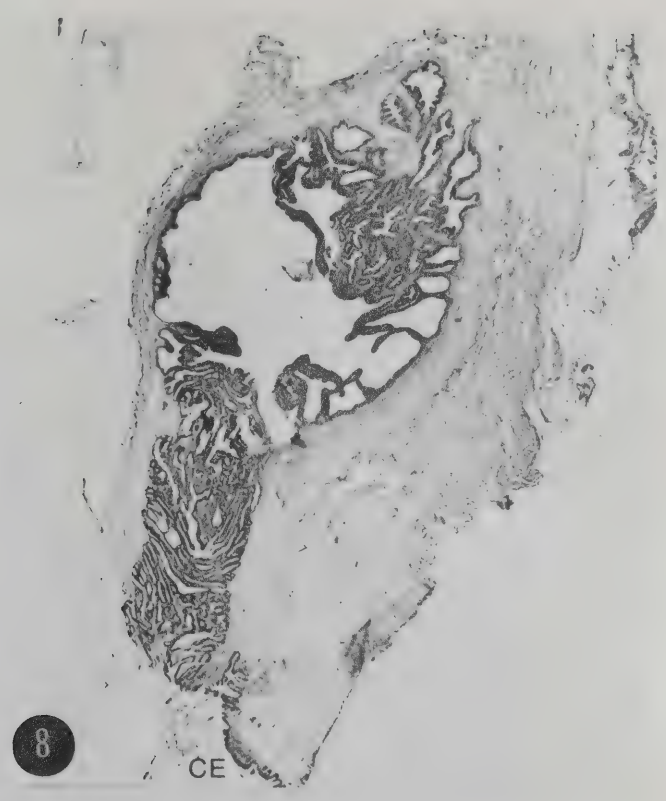
6



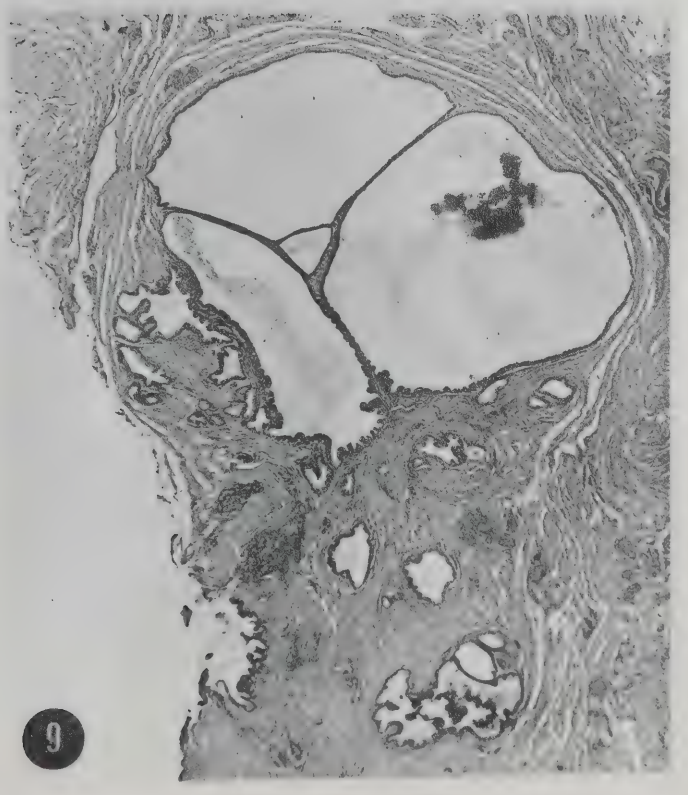
7

Figure 8 - Sagittal section of 10½ month bursa. Hematoxylin eosin. X 9. CE : cloacal epithelium

Figure 9 - Cross section of 15 month bursa. Hematoxylin eosin. X 24.



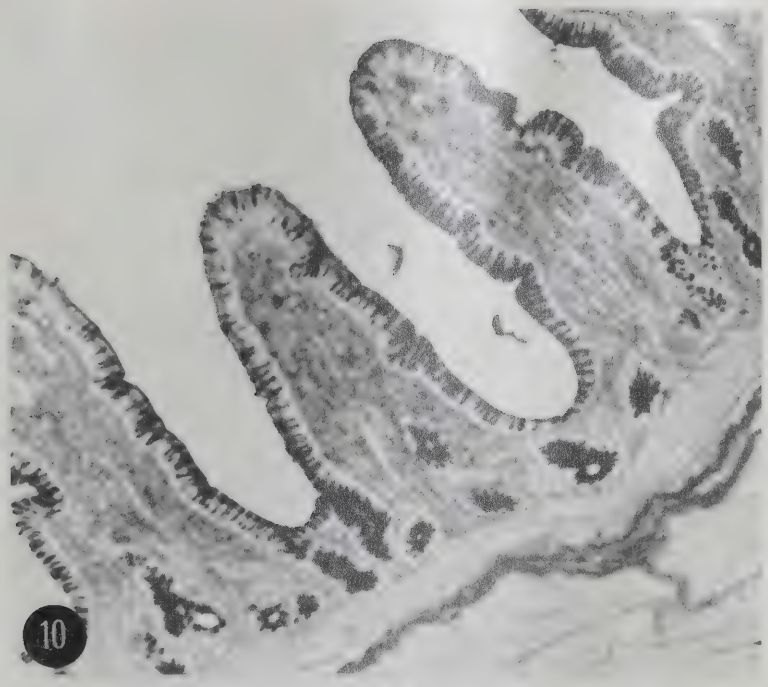
8



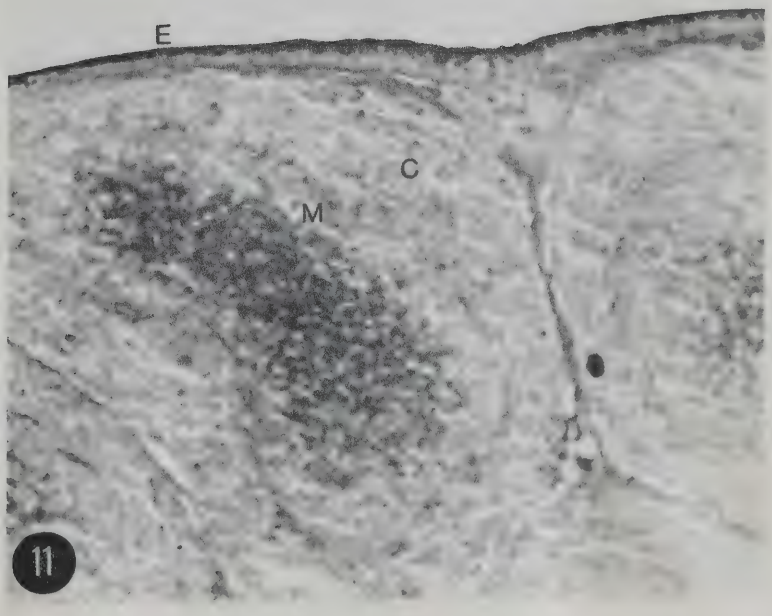
9

Figure 10 - Section of 13 week old coprodeal (cloacal) epithelium. Alcian blue-PAS stain showing intense alcianophilia of numerous goblet cells. X 176

Figure 11 - Section of 13 week old bursa epithelium. Alcian blue-PAS. Alcianophilia is restricted to surface coat and distal areas of cells. X 220. E : epithelium C : cortex M : medulla



10



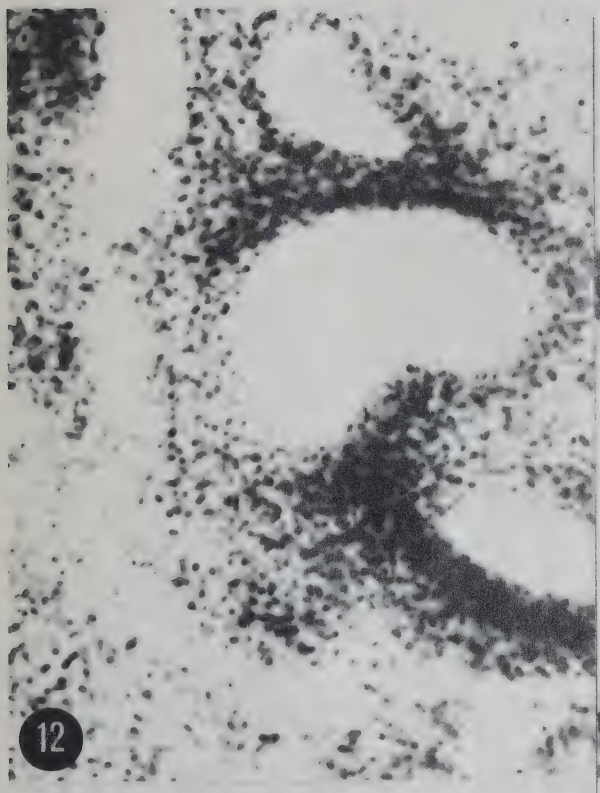
11

Figure 12 - Acid phosphatase activity in 4 year old persisting epithelium. Alpha-naphthol-phosphate method. X 750

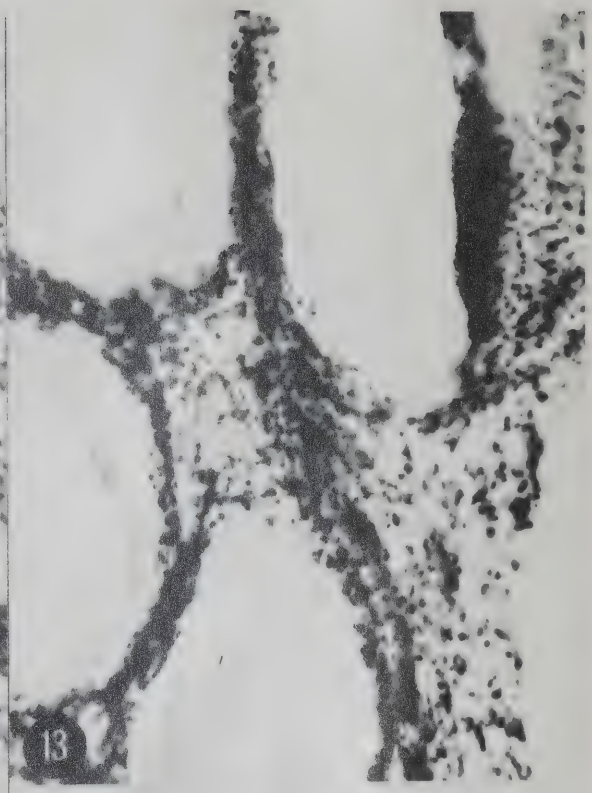
Figure 13 - Alkaline phosphatase activity in same tissue as preceding figure. Alpha-naphthol-phosphate method. X 750

Figure 14 - Section of epithelium and portion of lymphoid follicle of 13 week bursa. Azure A pH 3.9. X 600

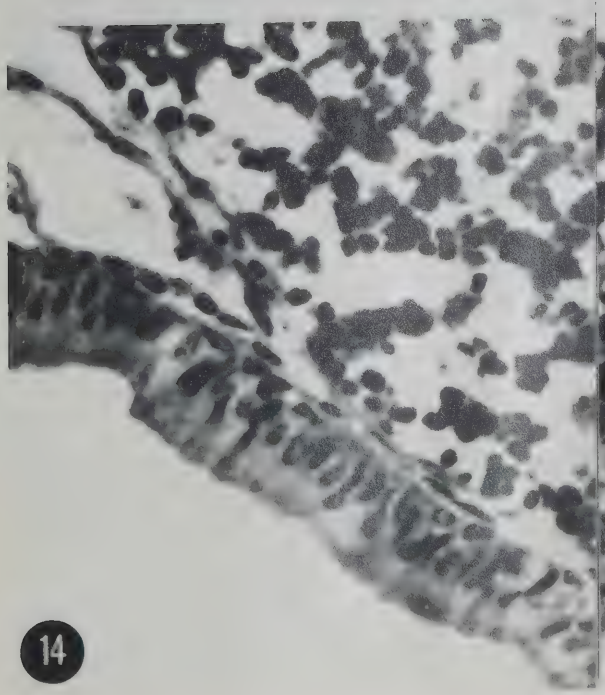
Figure 15 - Section of same bursa as preceding figure. RNAse, Azure A pH 3.9. X 620



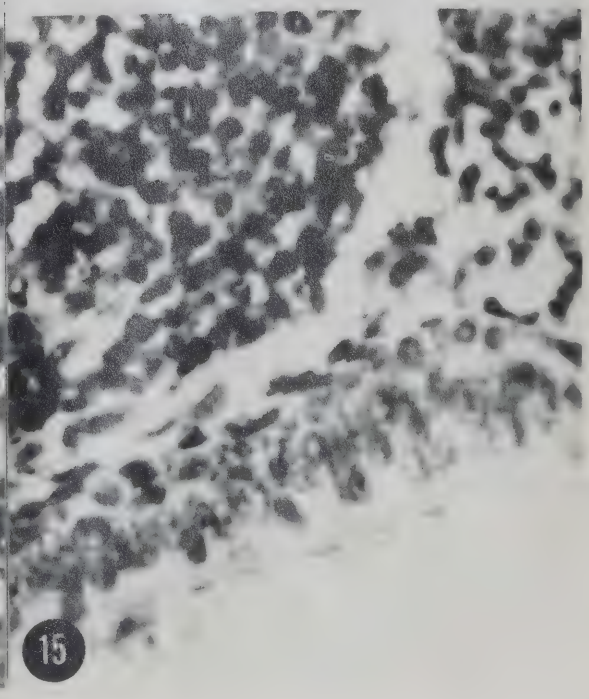
12



13



14



15

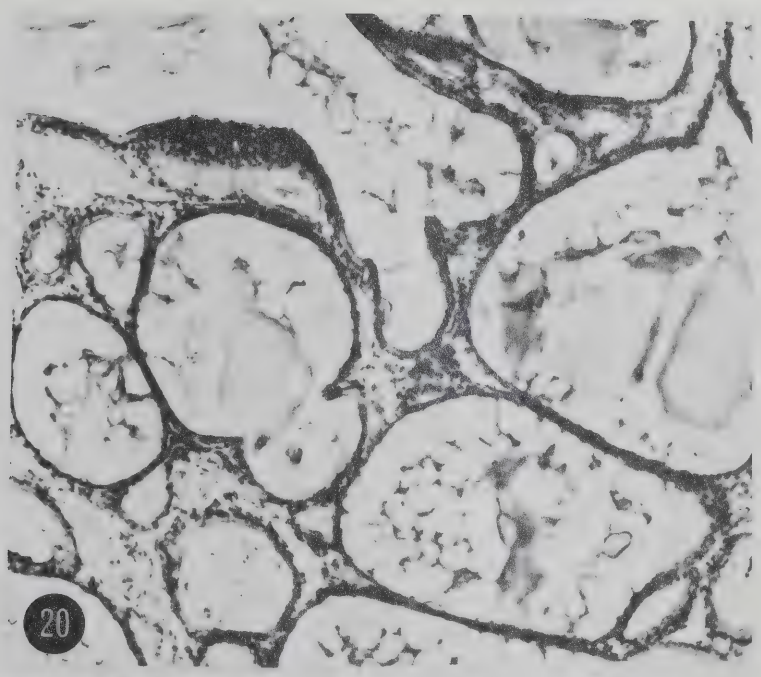
Figure 16 - Epithelium of 6 week bursa. Hematoxylin eosin. X 1760

Figure 17, 18 and 19 - Persisting epithelium from 15 month old bursas. Hematoxylin eosin. X 1540

16
17
18
19

Figure 20 - Cyst epithelium of a 15 month bursa. Hematoxylin eosin. X 176.

Figure 21 - Persisting epithelium and cyst epithelium in a 13 month bursa. Hematoxylin eosin. X 950. C : cyst epithelium
P : persisting epithelium


20

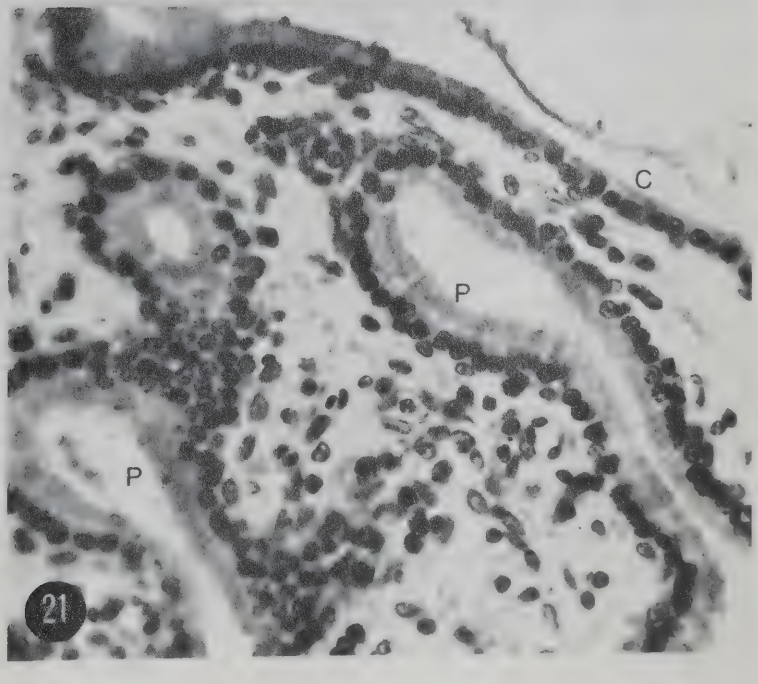

C
P
P
21

Figure 22 - Section of 12 week epithelium showing part of basal area and the typical predominant nuclear morphology at this age. Osmium, epon, uranyl acetate, lead citrate. X 9,000.

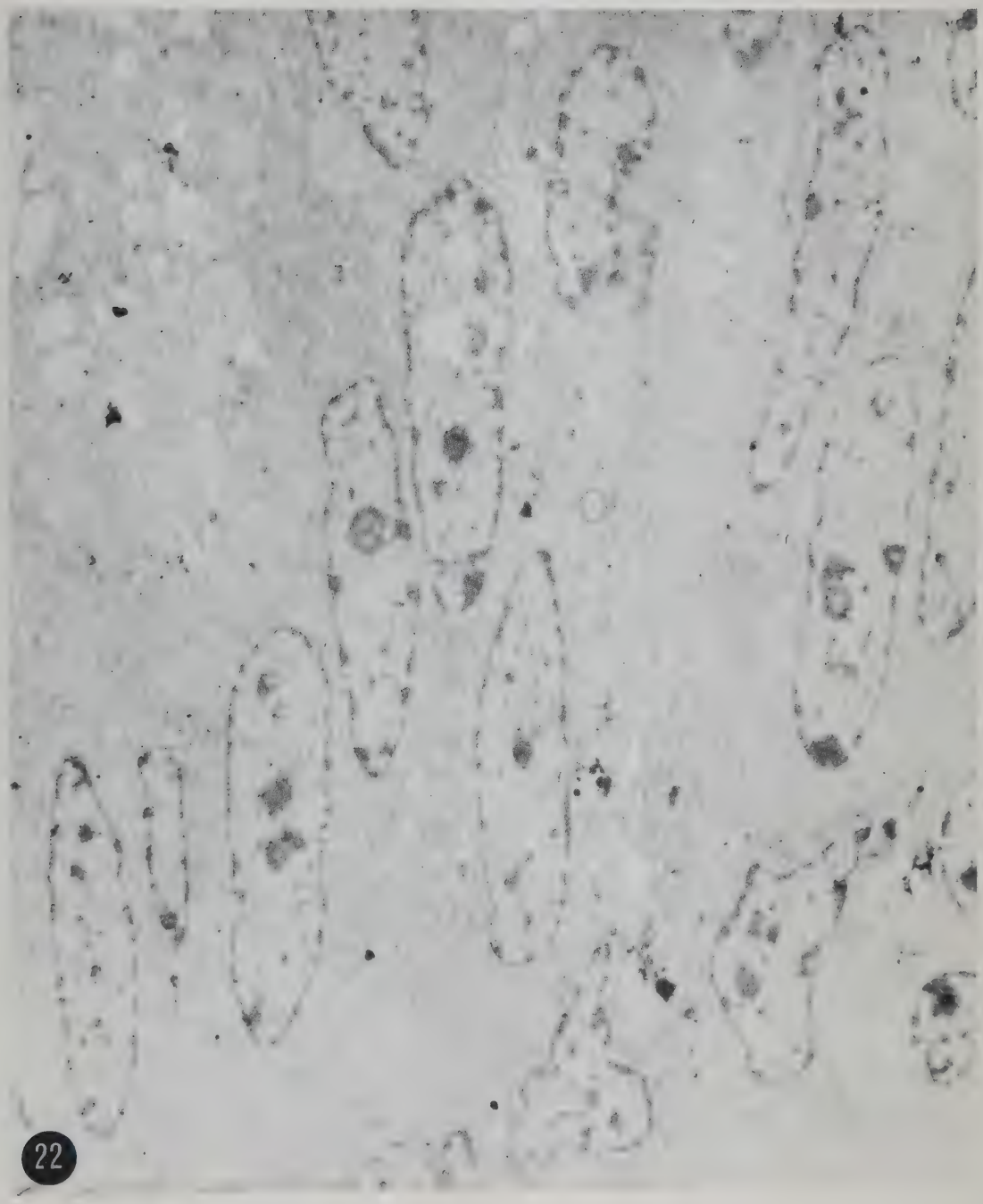

22

Figure 23 - Portion of secretory cell of 12 week bursa epithelium. This cell shows features which are common to persisting epithelial cells: interdigitations, terminal bar, Golgi apparatus and secretory materials. Osmium, araldite, uranyl acetate, lead citrate.

X 36,000

G : golgi

M : mitochondria

V : multivesicular bodies

I : interdigitation of cell membranes

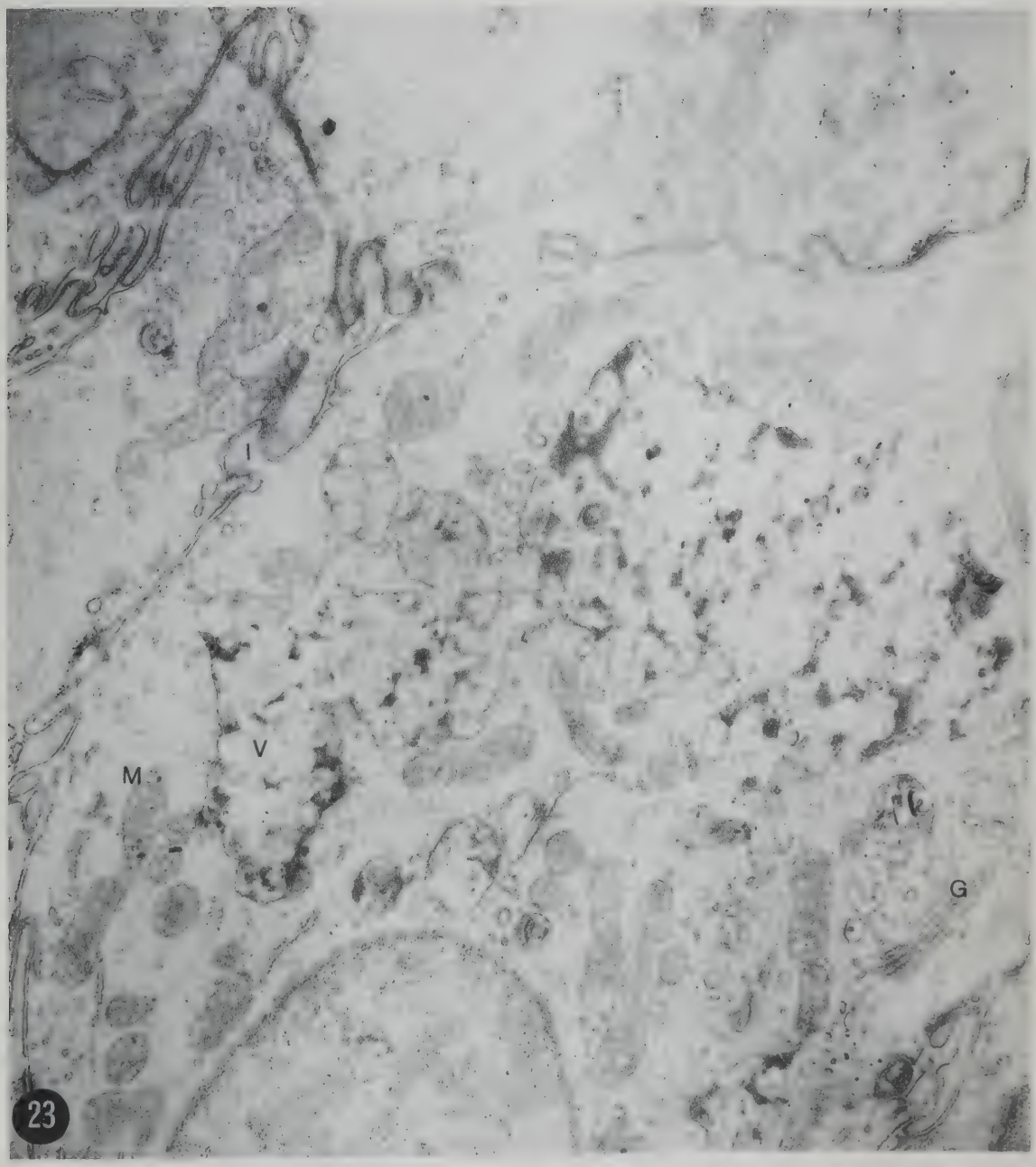

I
V
M
G
23

Figure 24 - This micrograph shows lumen surface details of two epithelial cells of a 12 week bursa. Osmium, araldite, uranyl acetate and lead citrate.

X 87,600 S : Secretory material

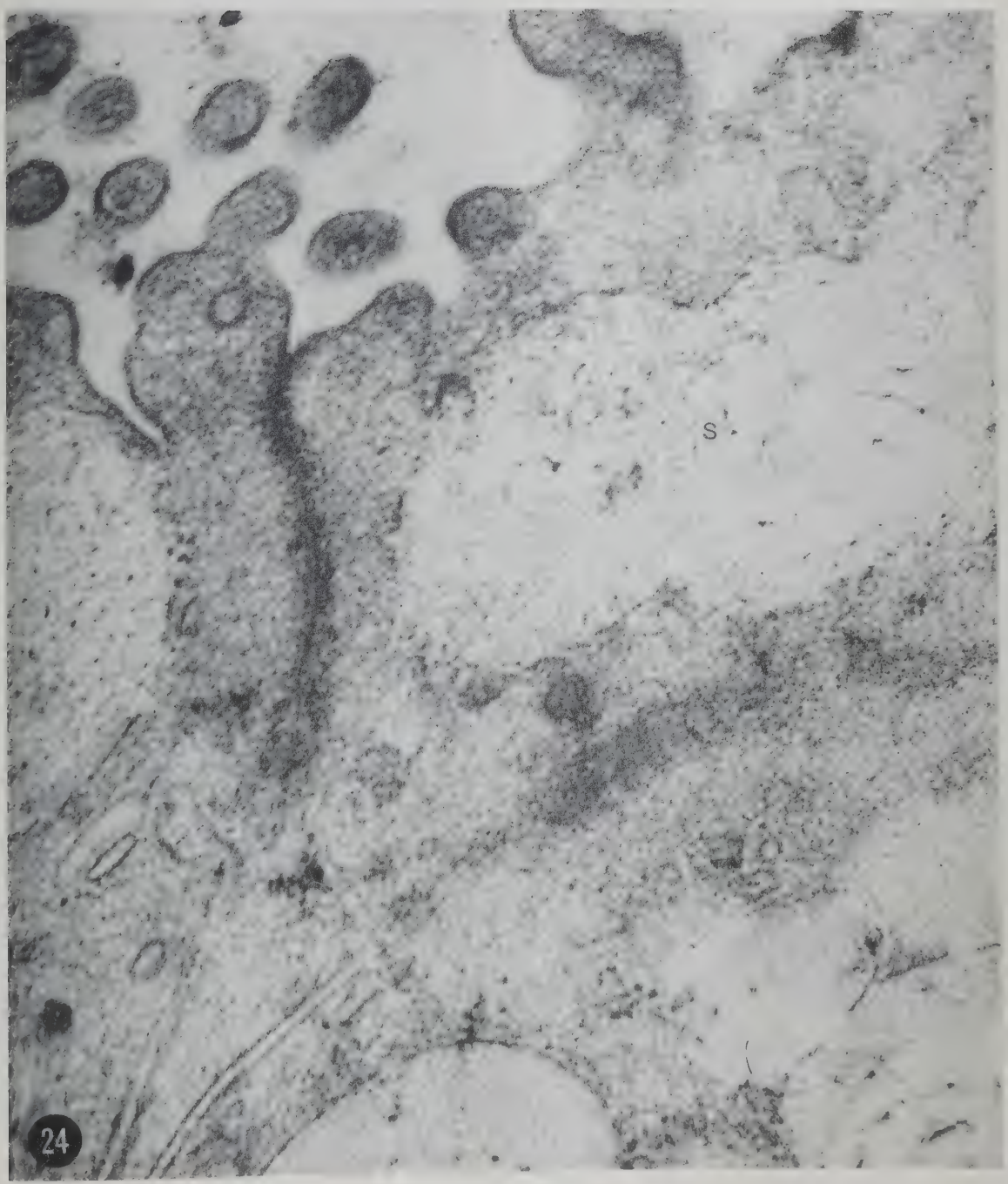
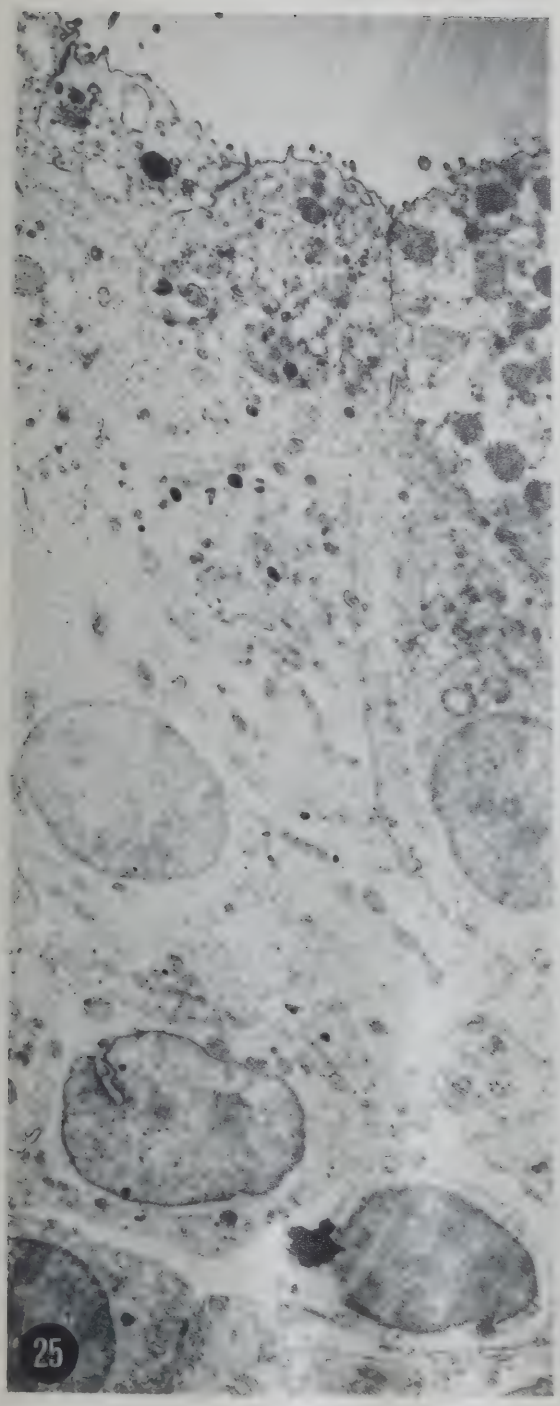

S
24

Figure 25 - Epithelial and basal cells of 26 week bursa. Osmium, araldite, uranyl acetate and lead citrate. X 9,000

Figure 26 - This shows an enlarged view of an epithelial cell in the previous micrograph. The features described in 12 week epithelial cells are apparent in this cell. Osmium, araldite, uranyl acetate and lead citrate. X 12,000


25

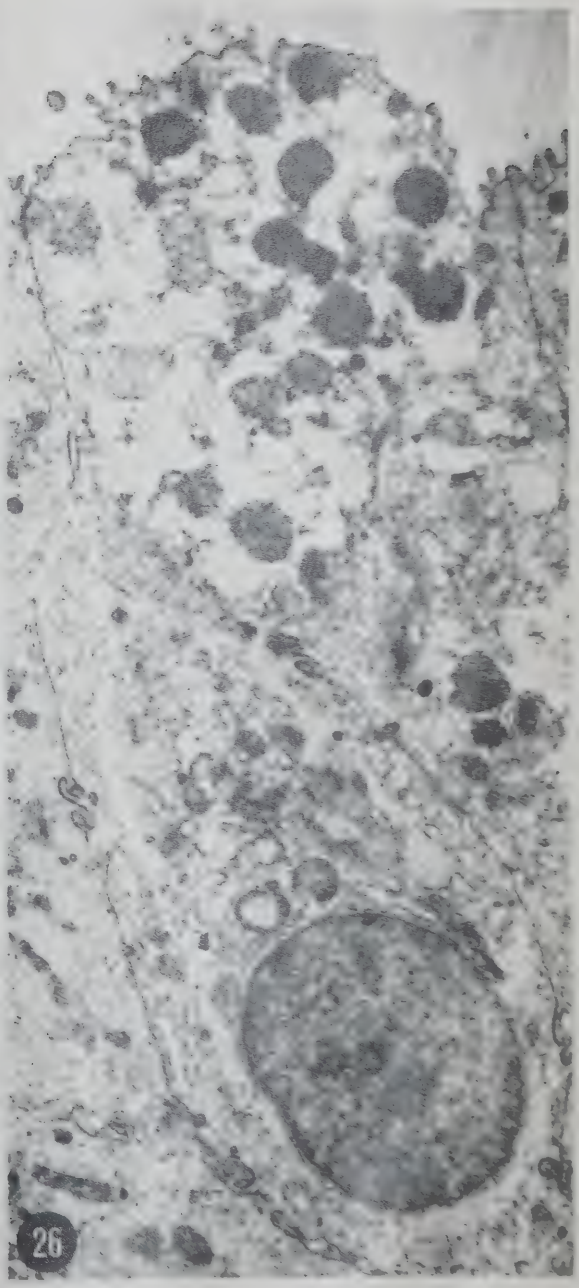

26

Figure 27 - Lumen surface details of 26 week epithelial cells. Osmium, araldite, uranyl acetate and lead citrate. X 25,320

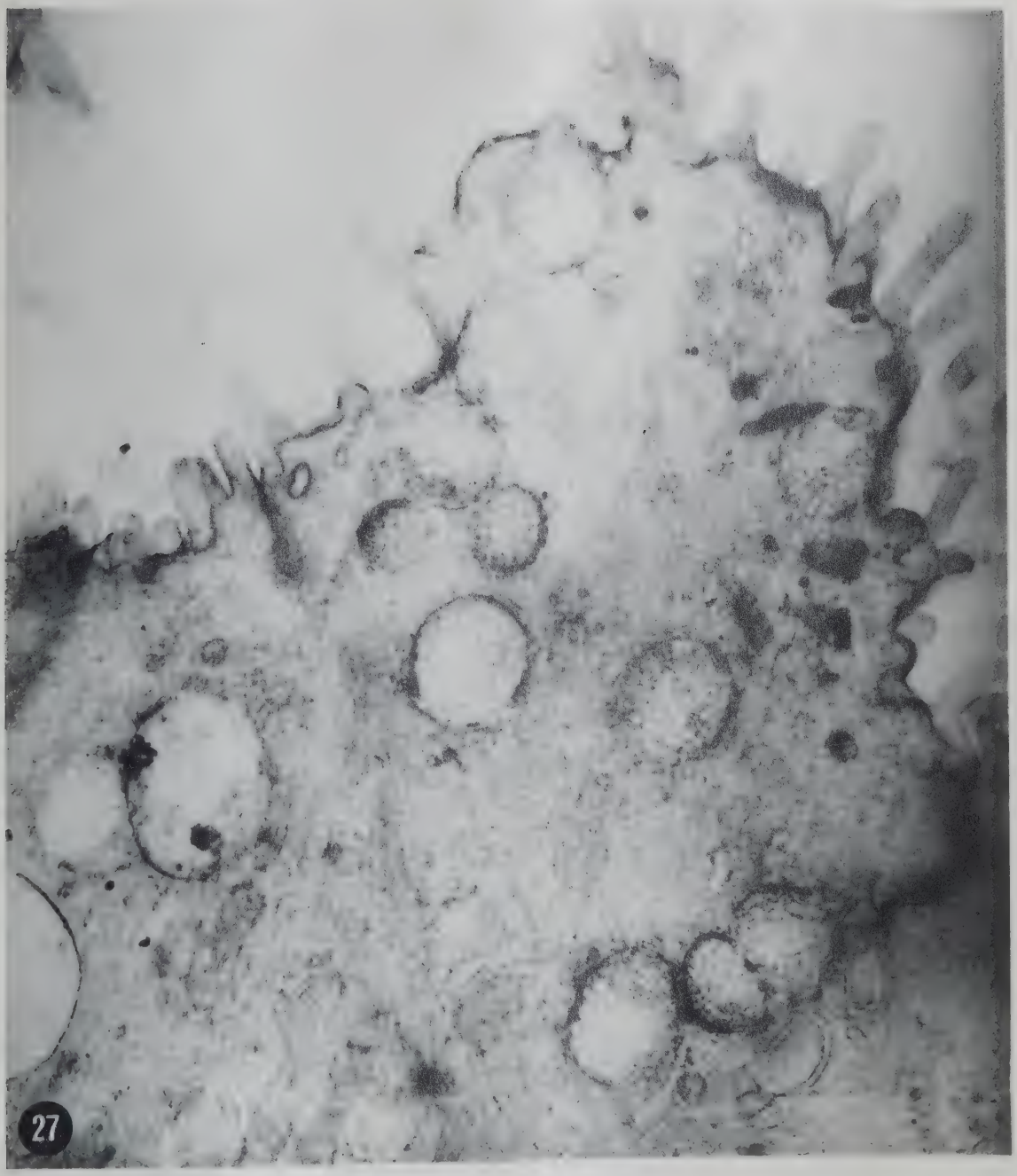
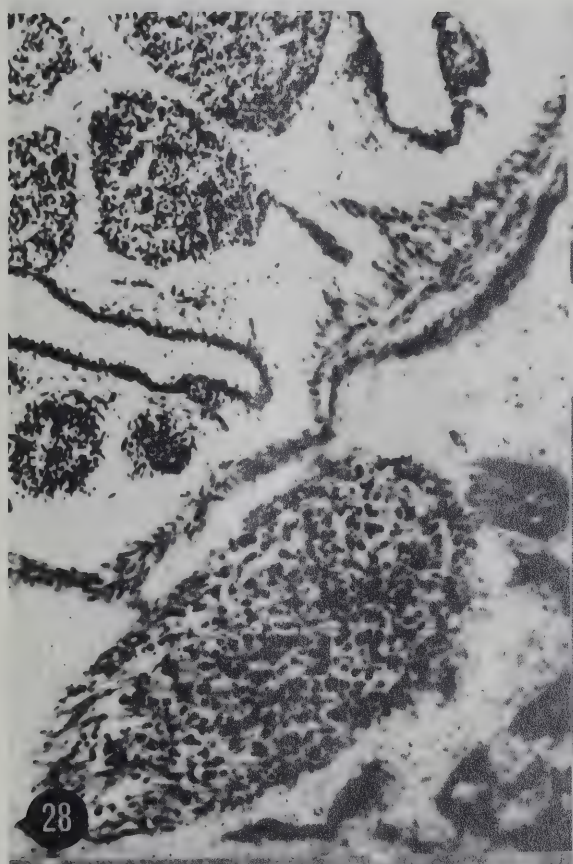

27

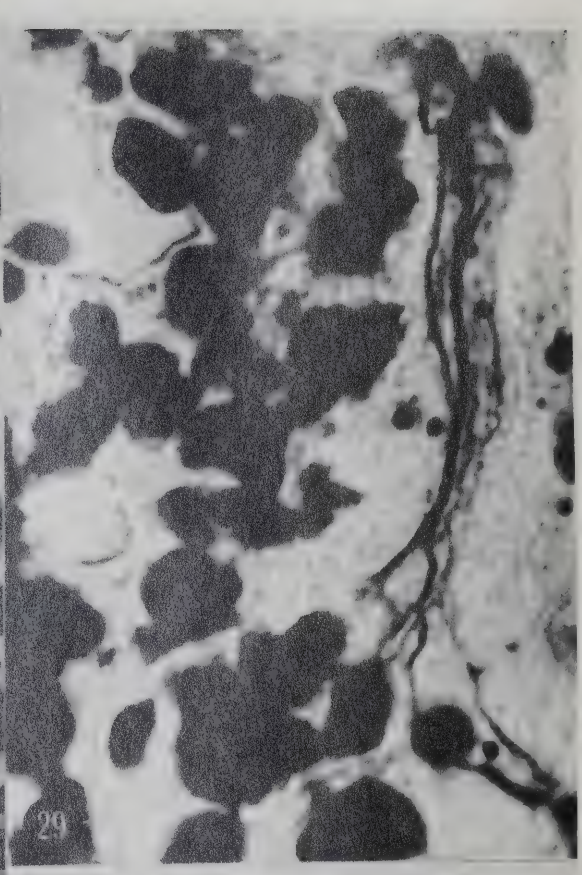
Figure 28 - Autonomic (parasympathetic) ganglion at base of 20 day embryonic bursa. Holmes silver impregnation. X 186.

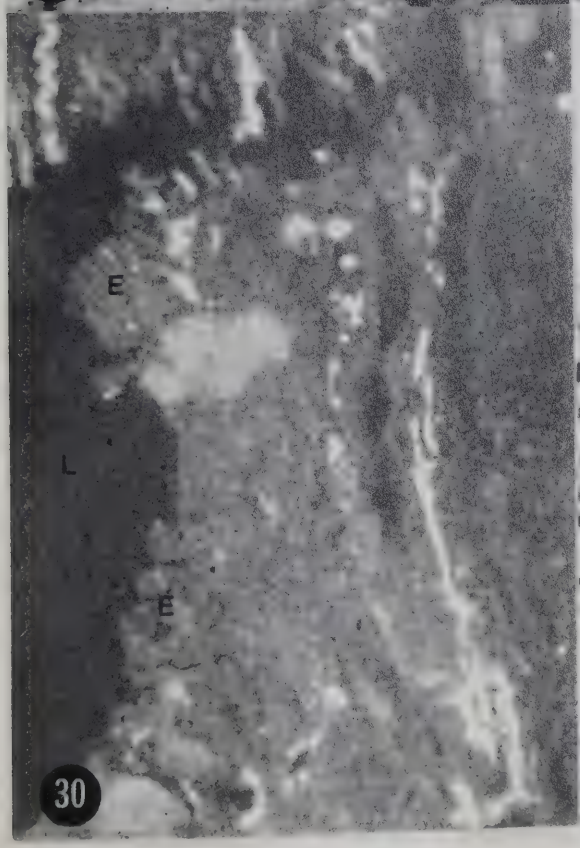
Figure 29 - Neurons approaching developing lymphoid follicle cortex in a 20 day embryonic bursa. Holmes silver impregnation. X 2520.

Figure 30 - Adrenergic fibres in periphery of 27 week old bursa. Falck paraformaldehyde fluorescence method. X 176.
L : lumen E : epithelium

Figure 31 - Demonstration of nerve fibres near persisting epithelium of 17 month bursa by methylene blue uptake. The epithelial cells are post stained with Azure A at pH 3.9. X 234.


28


29



30


31

Figure 32 - Neurons close to and approaching persisting epithelium in a 15 month bursa. Holmes silver impregnation. X 500

Figure 33 - Reticulin fibres in tunica propria of 8½ month bursa Gomori reticulin method. X 960

Figure 34 - Reticulin fibres in 15 month bursa. Gomori. X 460.
This figure and the preceding figures serve to demonstrate the difference between neurons and reticulin fibres and that Holmes impregnation does demonstrate neurons and not reticulin fibres.

32.

33

34

Figure 35 - Each point represents the mean grain count per cell of at least 500 cells in each of 5 bursa fragments. The fragments were labelled in short term tissue culture. Because the counts were made of areas of cells, both labelled and unlabelled cells are included. Although the labelling was weak and largely attributed to non-specific surface absorption (text, page 17) this figure was included because of the similarity of the labelling pattern to that obtained through intravenous administration of isotope in vivo (Figure 36).

3 WEEK BURSA
20 WEEK BURSA
GRAINS PER CELL
URIDINE-3H
THYMIDINE-3H
1.0
0.5
0.4
0.3
0.2
0.1
0.0
RNASE
EPITHELIUM
CORTEX
MEDULLA

Figure 36 - This figure represents grain counts from autoradiography of H-^{3} uridine and H^3 thymidine incorporation in 13 week old and 28-29 week old bursas. In this attempt the isotope was either injected directly into the bursa or intravenously or both. Each point represents the mean grain count per cell in at least 100 cells in 10 adjacent sections. Each box represents one bursa. The experiment was comprised of 5 birds.

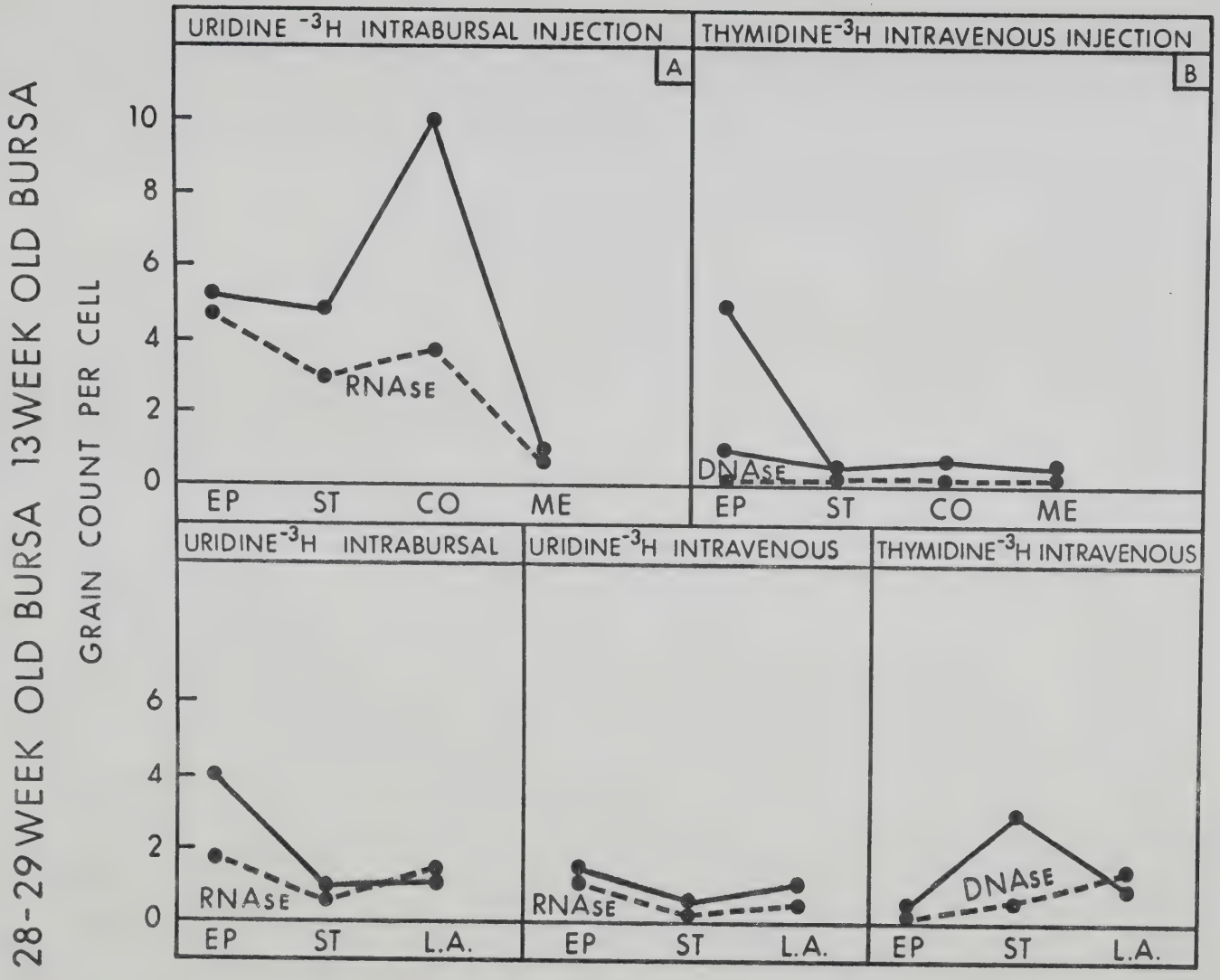
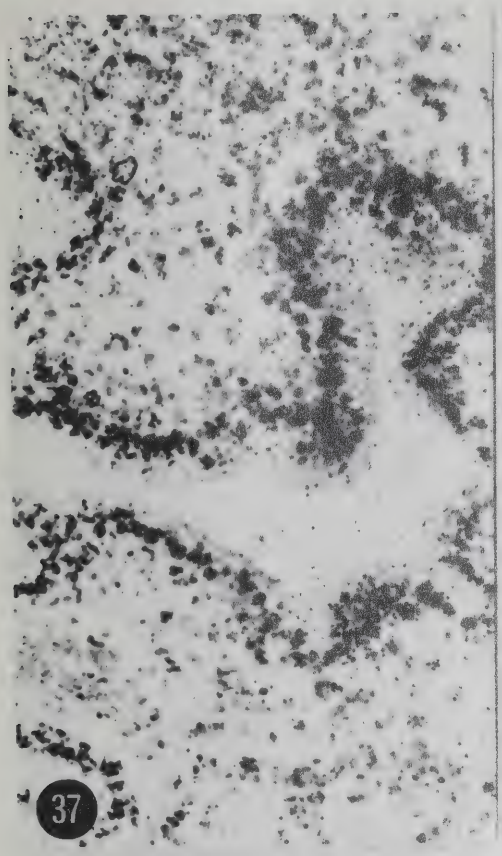

28-29 WEEK OLD BURSA 13 WEEK OLD BURSA
GRAIN COUNT PER CELL
URIDINE -3H INTRABURSAL INJECTION
A
THYMIDINE-3H INTRAVENOUS INJECTION
B
10
8
6
4
2
0
RNASE
DNASE
EP ST CO ME
URIDINE-3H INTRABURSAL
URIDINE-3H INTRAVENOUS
THYMIDINE-3H INTRAVENOUS
6
4
2
0
RNASE
RNASE
DNASE
EP ST L.A.
EP ST L.A.
EP ST L.A.

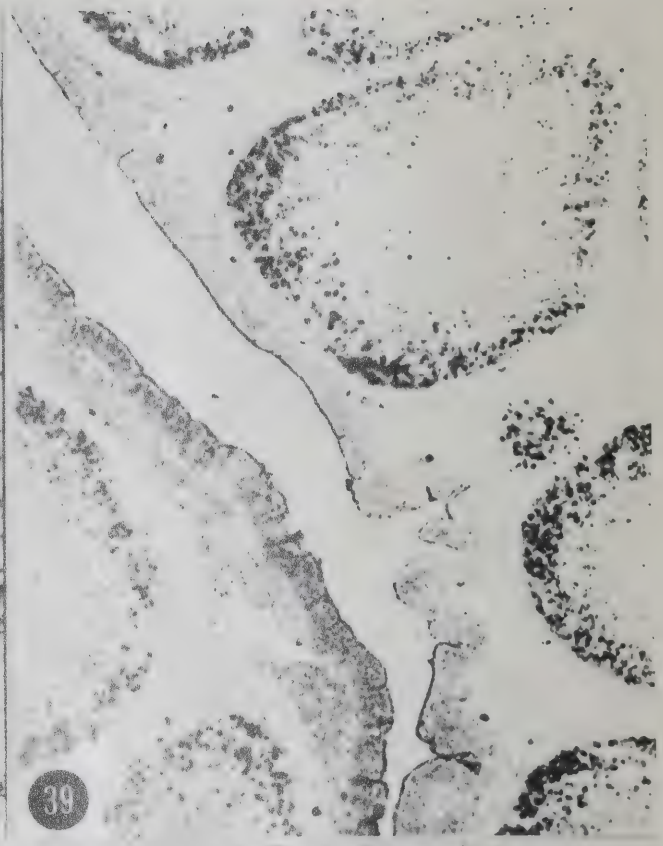
Figure 37 - Autoradiograph of H^3 -uridine incorporation in 29 week old bursa. 50 micro Curies were injected into the lumen of the bursa. Labelling was for 90 minutes. Autoradiographs were exposed for 3 weeks. Hematoxylin eosin. X 350.

Figure 38 - Autoradiograph of H^3 -uridine incorporation in 29 week old bursa. Conditions were identical to the previous autoradiograph with the addition of RNAse digestion for 2 hours after labelling. Hematoxylin eosin. X 350.

Figure 39 - Autoradiographs of H^3 -uridine incorporation in 13 week bursa. 50 micro Curies were injected into the bursa lumen. Labelling was for 90 minutes. Exposure was 3 weeks. Hematoxylin eosin. X 160

Figure 40 - Autoradiograph of H^3 -uridine incorporation in a 28 week bursa. 850 micro Curies were injected intravenously and locally. Labelling was for 24 hours. Exposure was 3 weeks. Hematoxylin eosin. X 350


37


39


38

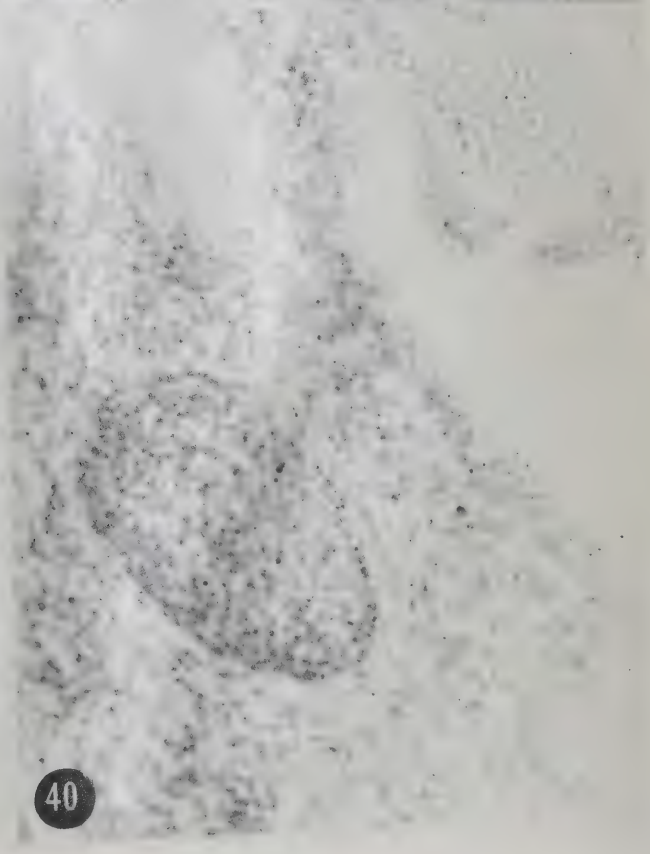
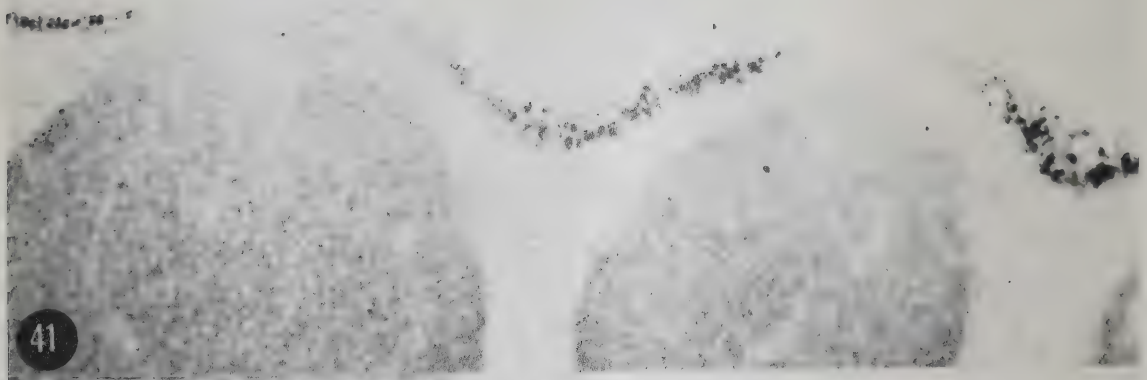

40

Figure 41 - Autoradiograph of H^3 -thymidine incorporation in a 13 week bursa. 50 micro Curies injected into the bursa lumen. Labelling for 90 minutes. Exposure for 3 weeks. Hematoxylin eosin. X 205

Figure 42 - Detailed view of part of epithelium and follicle of previous Figure. X 640

Figure 43 - Autoradiograph of H^3 -thymidine incorporation in 28 week old bursa. 850 micro Curies were injected intravenously and locally. Labelling was for 24 hours. Exposure was for 3 weeks. Hematoxylin eosin. X 600

Figure 44 - Detailed view of a degenerating lymphoid follicle from the same tissue as the previous autoradiograph. X 160


41


42
44

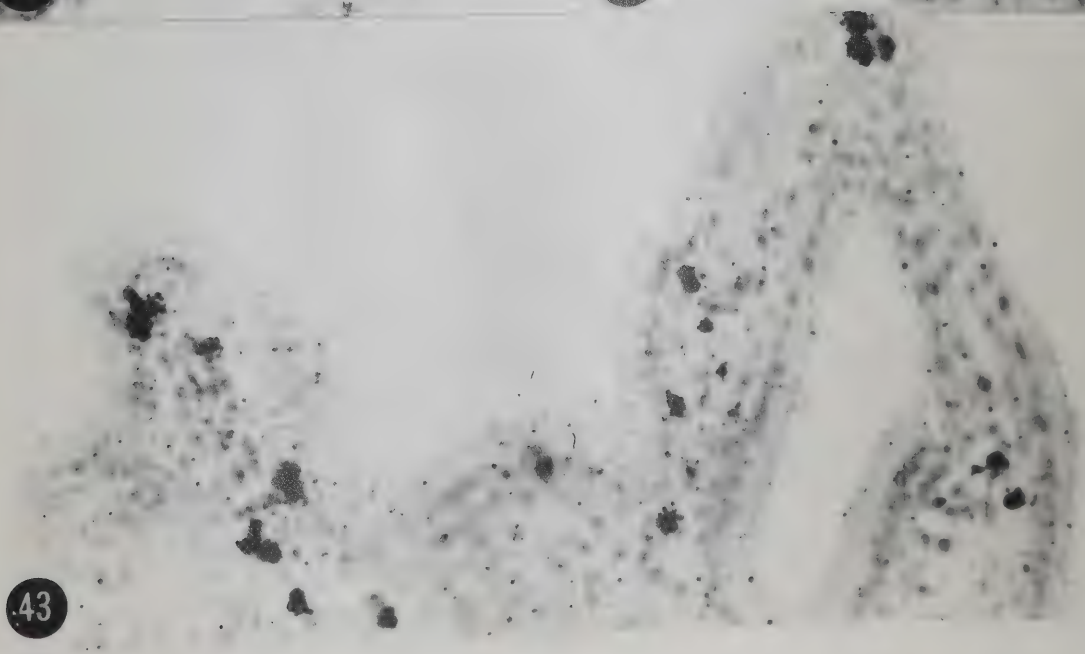

43

Figure 45 - Explanation of Figure.

The data for all 3 bursectomy-replacement experiments were pooled. The mean titre of natural antibody to rabbit erythrocytes of the control groups for each week of the experiments was calculated. The difference of each individual bursectomy or replacement titre from the control mean was calculated. The mean of the differences for each week was calculated and plotted. The lines were fitted according to the regression of the mean of the difference on days of age. The slopes of the lines were significantly different ($p < .01$) according to students t test. Standard errors were calculated for the means but were not displayed as they did not exceed $\pm$ 0.73 $\log_2$ units. The 7 week average of the standard errors for the B means was 0.47, for the B-B means, 0.33.

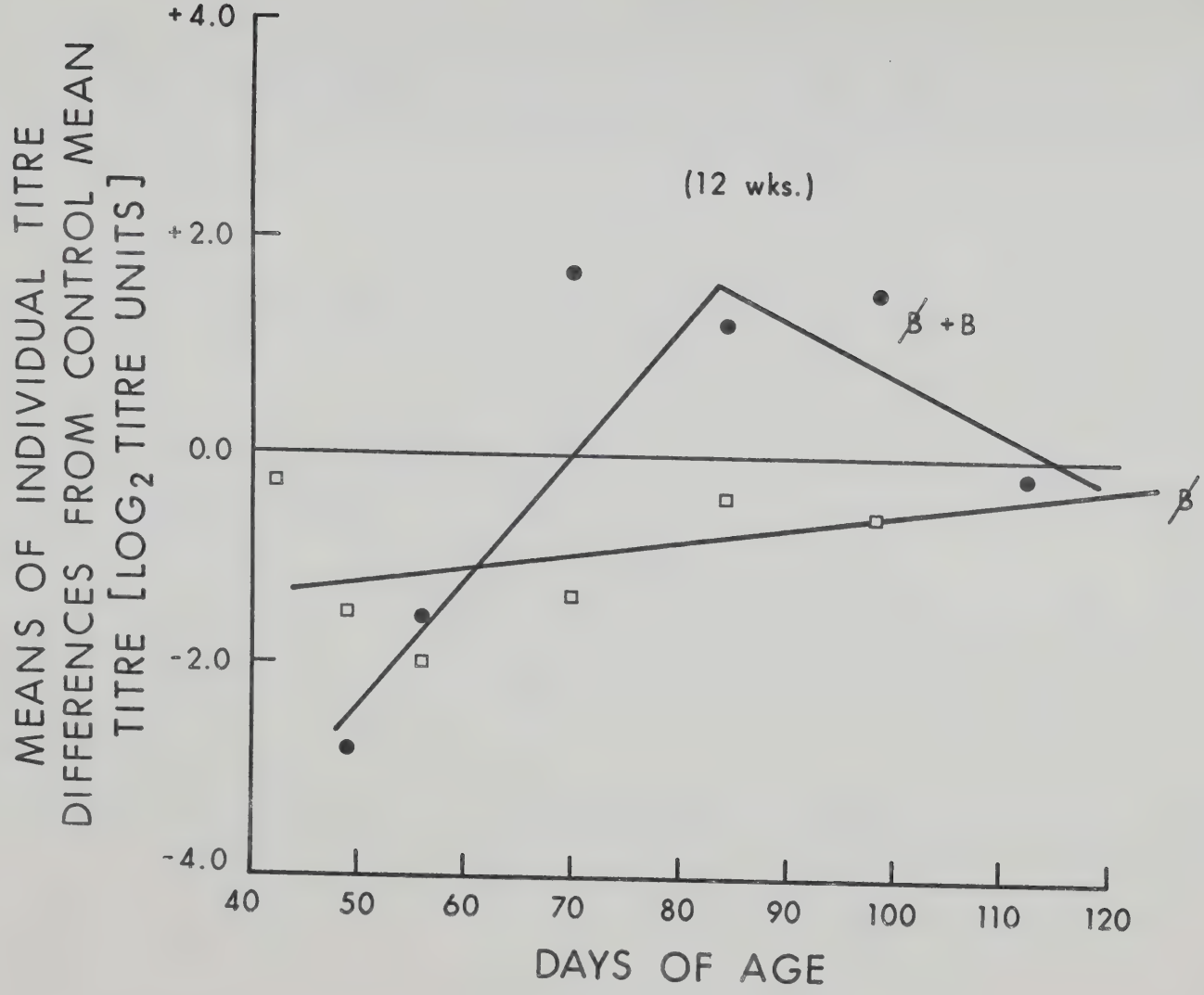

MEANS OF INDIVIDUAL TITRE DIFFERENCES FROM CONTROL MEAN TITRE [LOG2 TITRE UNITS]
+4.0
+2.0
0.0
-2.0
-4.0
(12 wks.)
β +B
β
40
50
60
70
80
90
100
110
120
DAYS OF AGE

Figure 46 - The proportion of antibody producing individuals in each group was calculated on the total number of individuals in the group. The lines were drawn from regression formulas. The total numbers of individuals in each group in this and the preceding figure were; control 20, bursectomy 18, replacement 22.

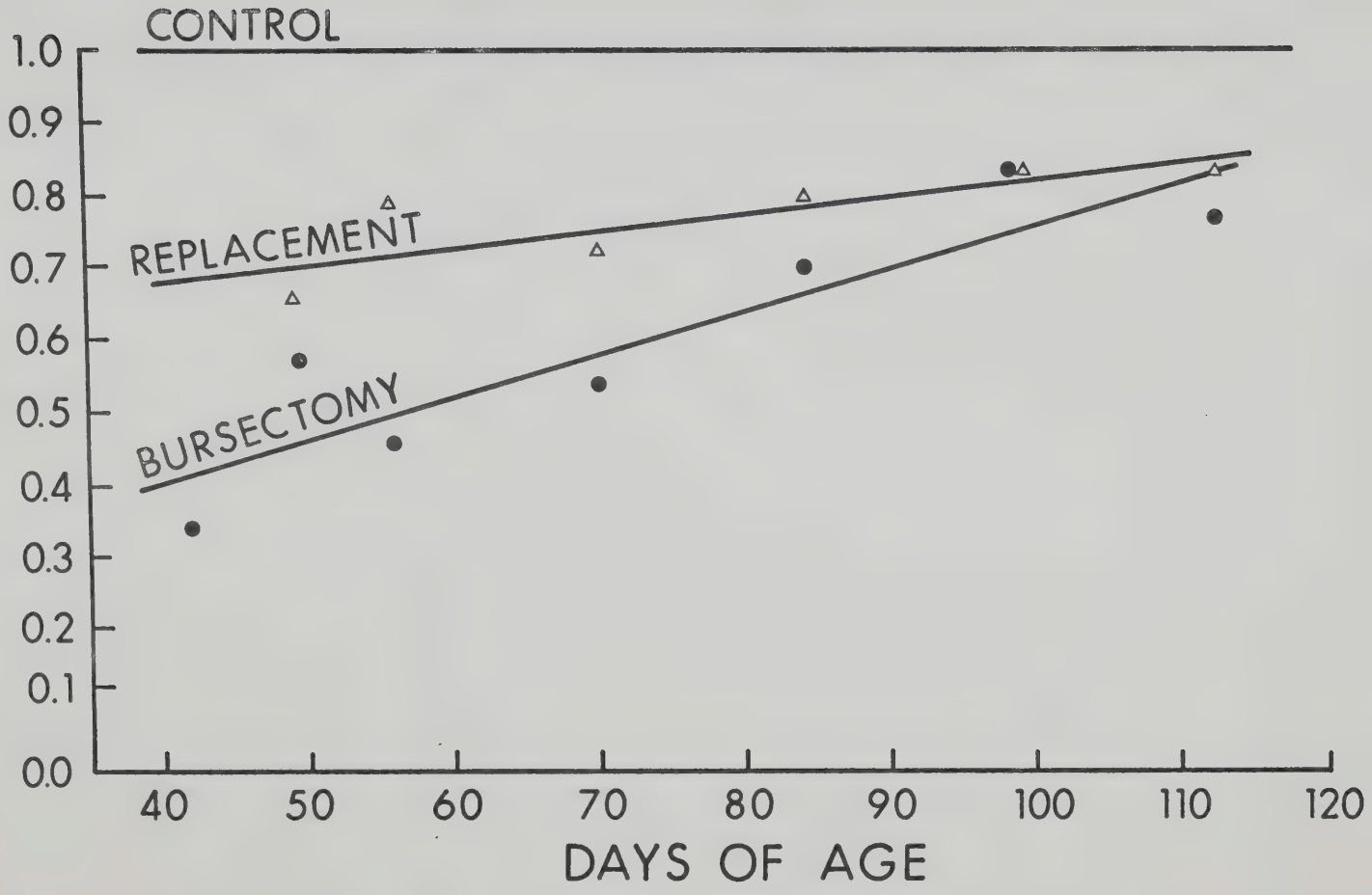

CONTROL
REPLACEMENT
BURSECTOMY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
40
50
60
70
80
90
100
110
120
DAYS OF AGE

Figure 47 - Frequency distributions of titres of all birds in each experimental group at 8 and 16 weeks after the operations. The curves are only drawn to approximately fit the points in view of the low numbers of each group; control 20, bursectomy 18, replacement, 22. — — — 8 wk.
------ 16 wk.

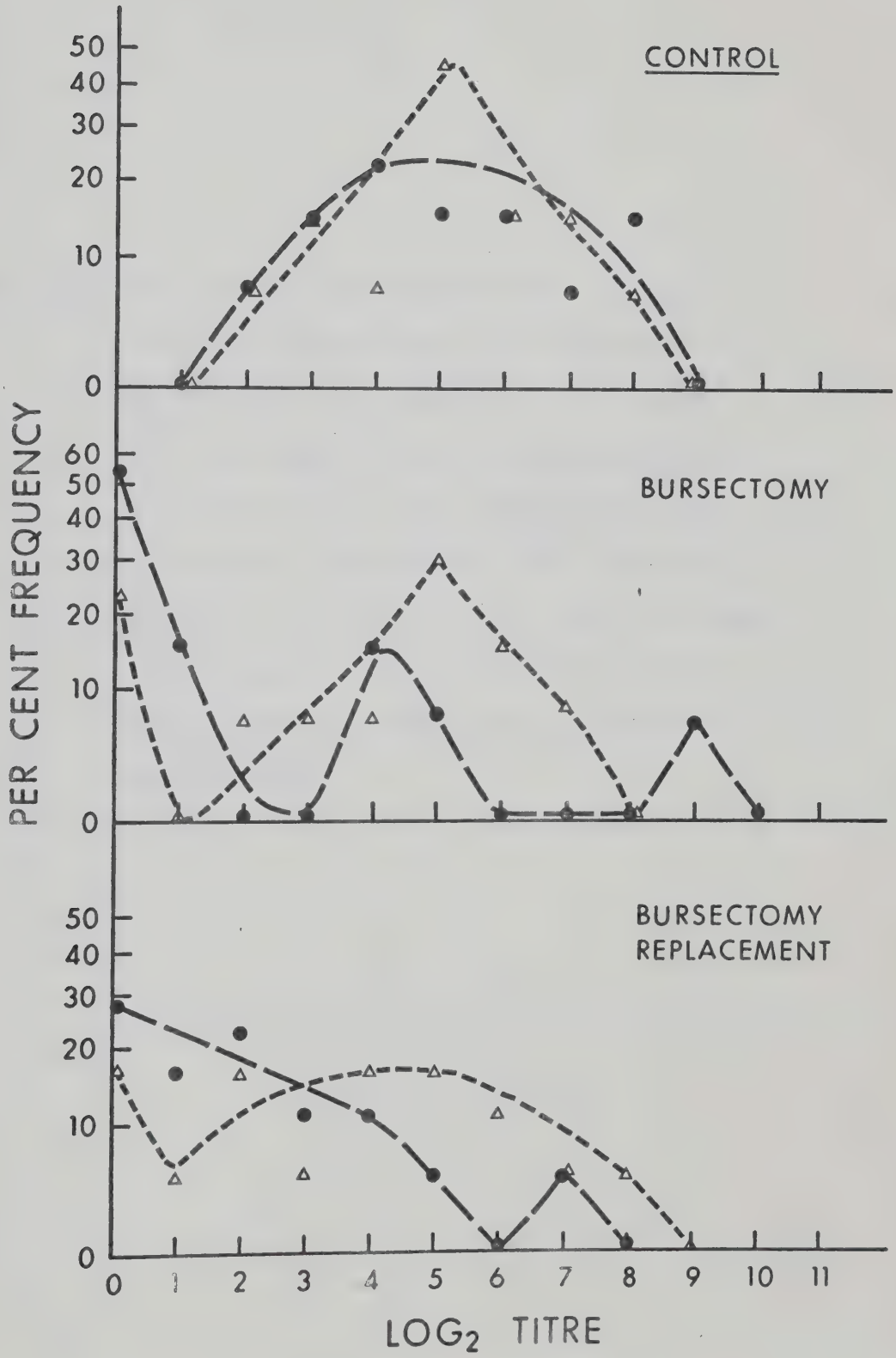

CONTROL
BURSECTOMY
BURSECTOMY
REPLACEMENT
PER CENT FREQUENCY
LOG2 TITRE
0
10
20
30
40
50
60
0 1 2 3 4 5 6 7 8 9 10 11

Figure 48 - Survival of commercial White Leghorns following bursectomy and replacement of the bursa. The numbers of each group and the mean survival in days are: control (31) 109.3 days, bursectomy (23) 79.4 days, replacement (22) 99.6 days. ($F_{2,\ 73}$ 44.27 ***). A t test of means demonstrated significant differences only between control and bursectomy means (p.05).

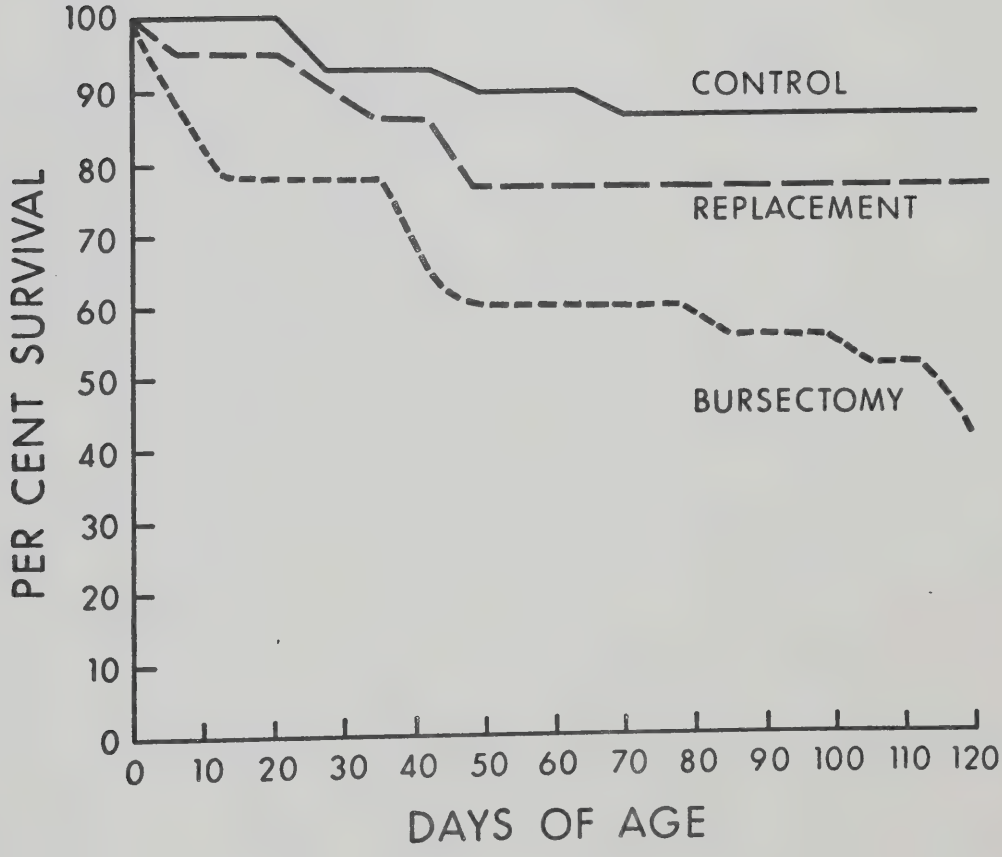

PER CENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90 100 110 120
DAYS OF AGE
CONTROL
REPLACEMENT
BURSECTOMY

Figure 49 - Natural agglutinin production and body weight in birds bursectomized or sham operated at fourteen weeks of age. Standard error is shown only where there is no overlap.

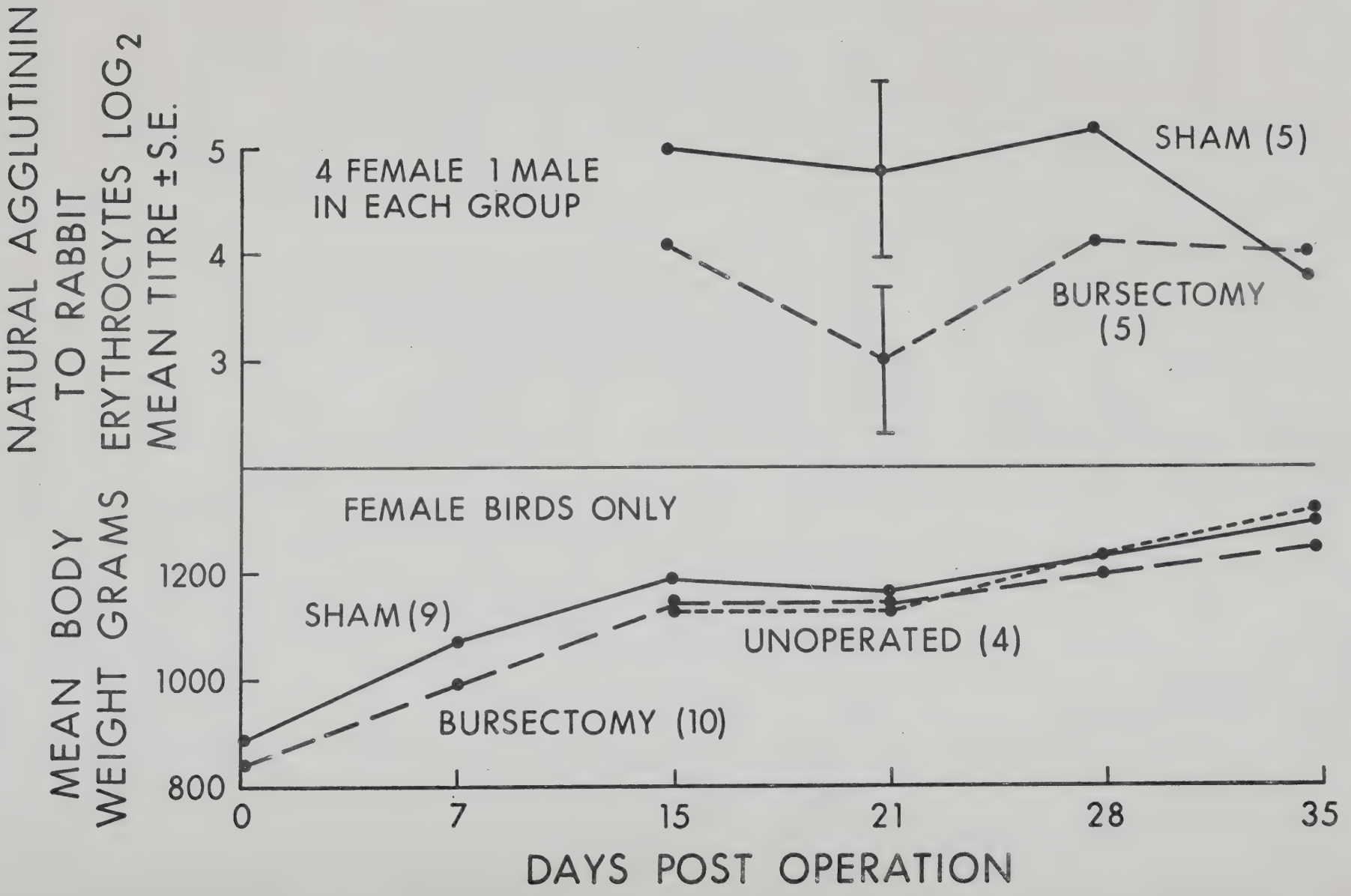

NATURAL AGGLUTININ TO RABBIT ERYTHROCYTES LOG2 MEAN TITRE ±S.E.
5
4
3
4 FEMALE 1 MALE IN EACH GROUP
SHAM (5)
BURSECTOMY (5)
MEAN BODY WEIGHT GRAMS
FEMALE BIRDS ONLY
1200
1000
800
SHAM (9)
UNOPERATED (4)
BURSECTOMY (10)
0
7
15
21
28
35
DAYS POST OPERATION

Figure 50 - Mean total peripheral leucocyte counts per cubic millimeter of blood in female birds bursectomized, sham operated or unoperated at fourteen weeks of age. Standard errors are indicated. Individual numbers are indicated. The four unoperated control females did not receive antibiotic treatment at fourteen weeks, all other individuals did.

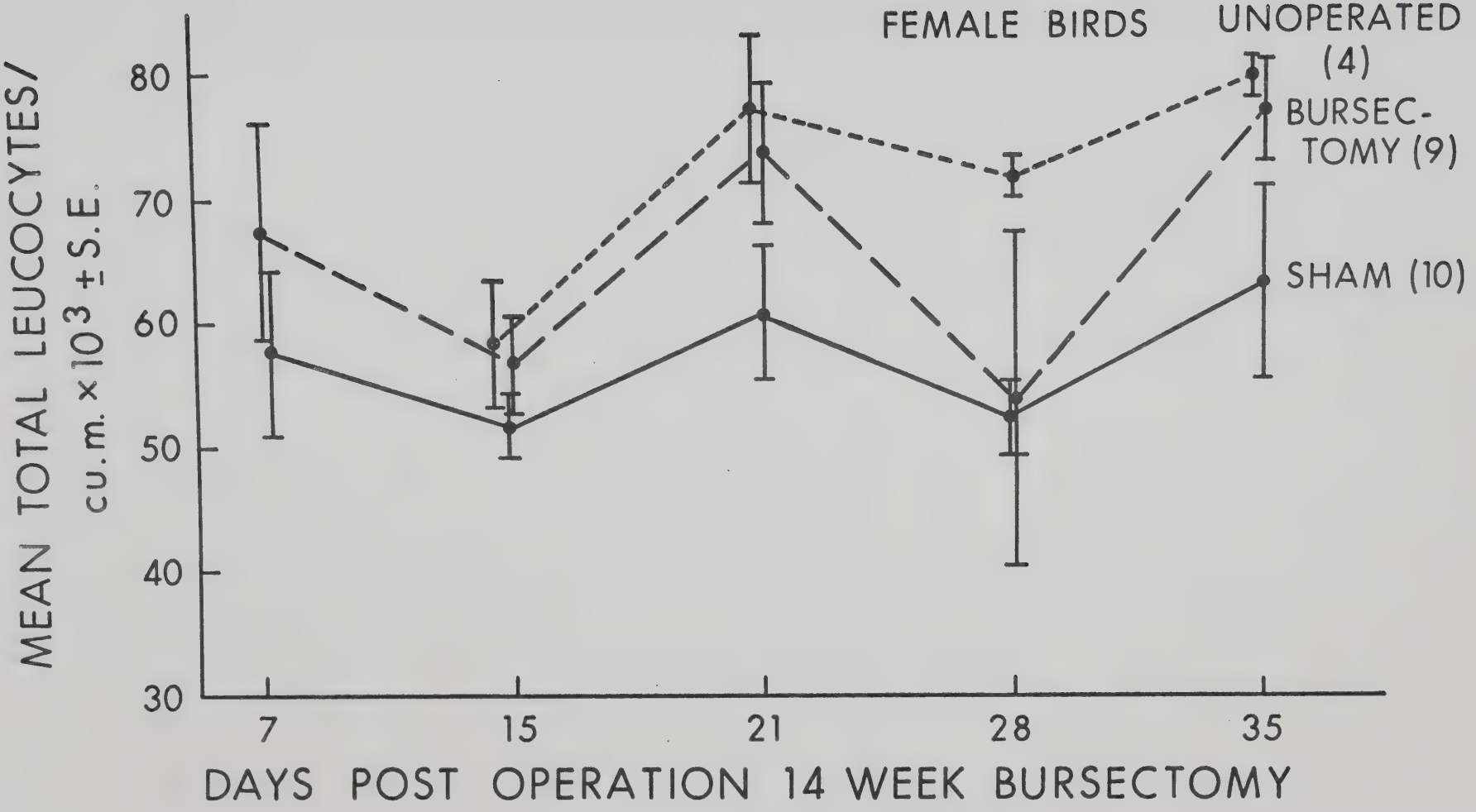

FEMALE BIRDS
UNOPERATED (4)
BURSEC-TOMY (9)
SHAM (10)
MEAN TOTAL LEUCOCYTES/ cu.m. x 10³ ± S.E.
80
70
60
50
40
30
7
15
21
28
35
DAYS POST OPERATION 14 WEEK BURSECTOMY

Figure 51 - Mean total peripheral leucocyte counts per cubic millimeter of blood in male birds sham operated or bursectomized at fourteen weeks. Standard errors and individual numbers are indicated.

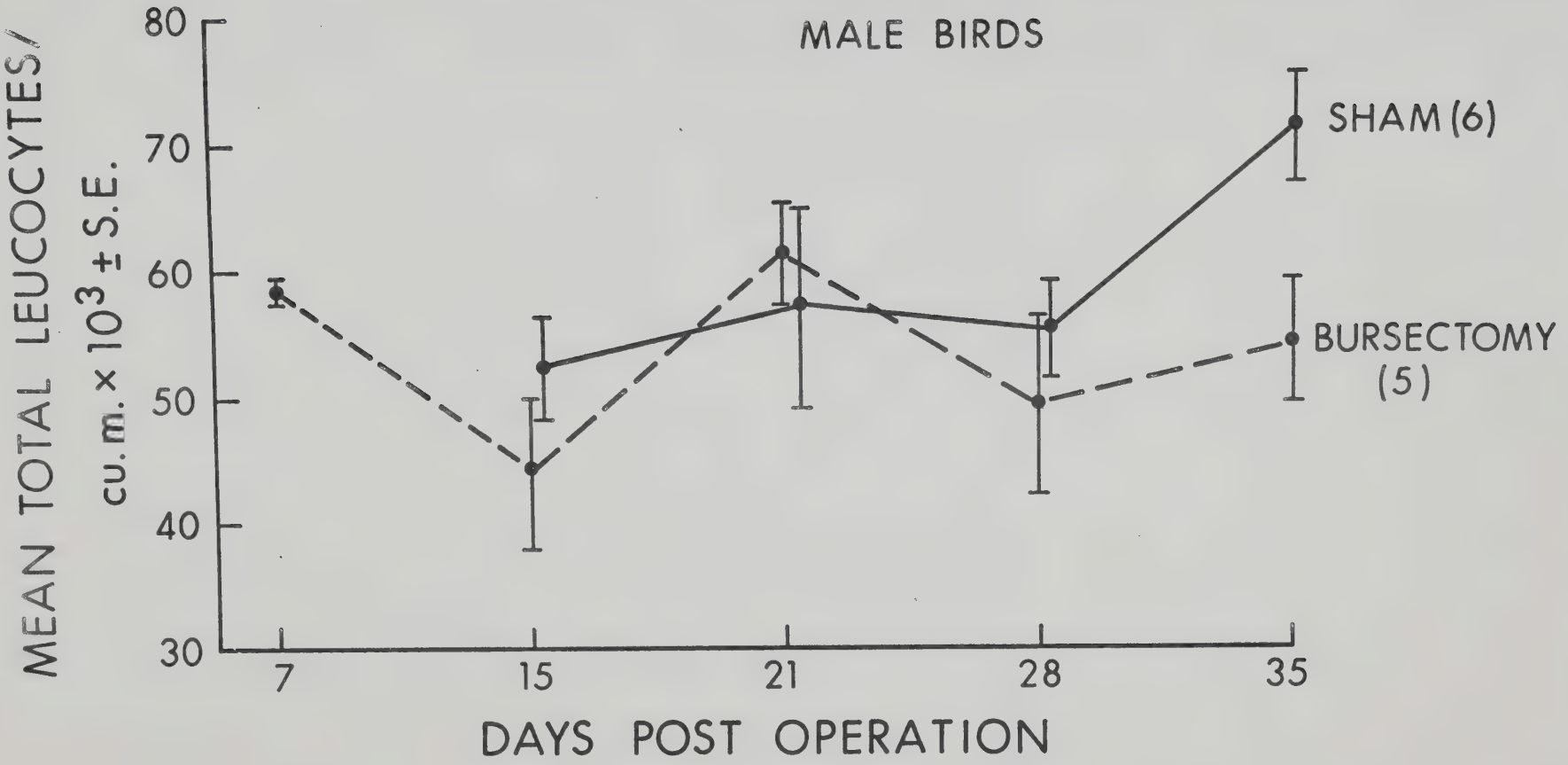

MALE BIRDS
MEAN TOTAL LEUCOCYTES/ cu.m. × 10³ ± S.E.
80
70
60
50
40
30
7
15
21
28
35
DAYS POST OPERATION
SHAM (6)
BURSECTOMY (5)

Figure 52 - Total peripheral leucocyte counts per cubic millimeter of blood in two bursectomized females and two sham operated females over a twenty four hour period.

Bursectomy -----,

sham operation _____

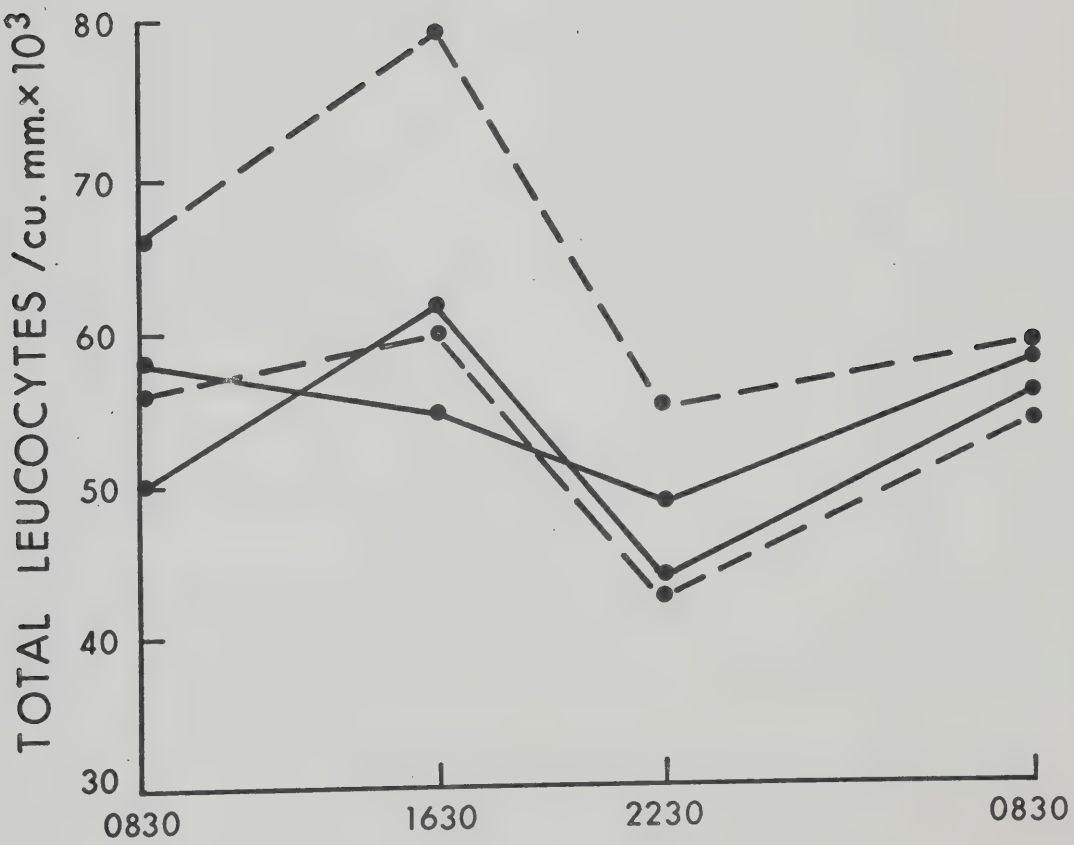
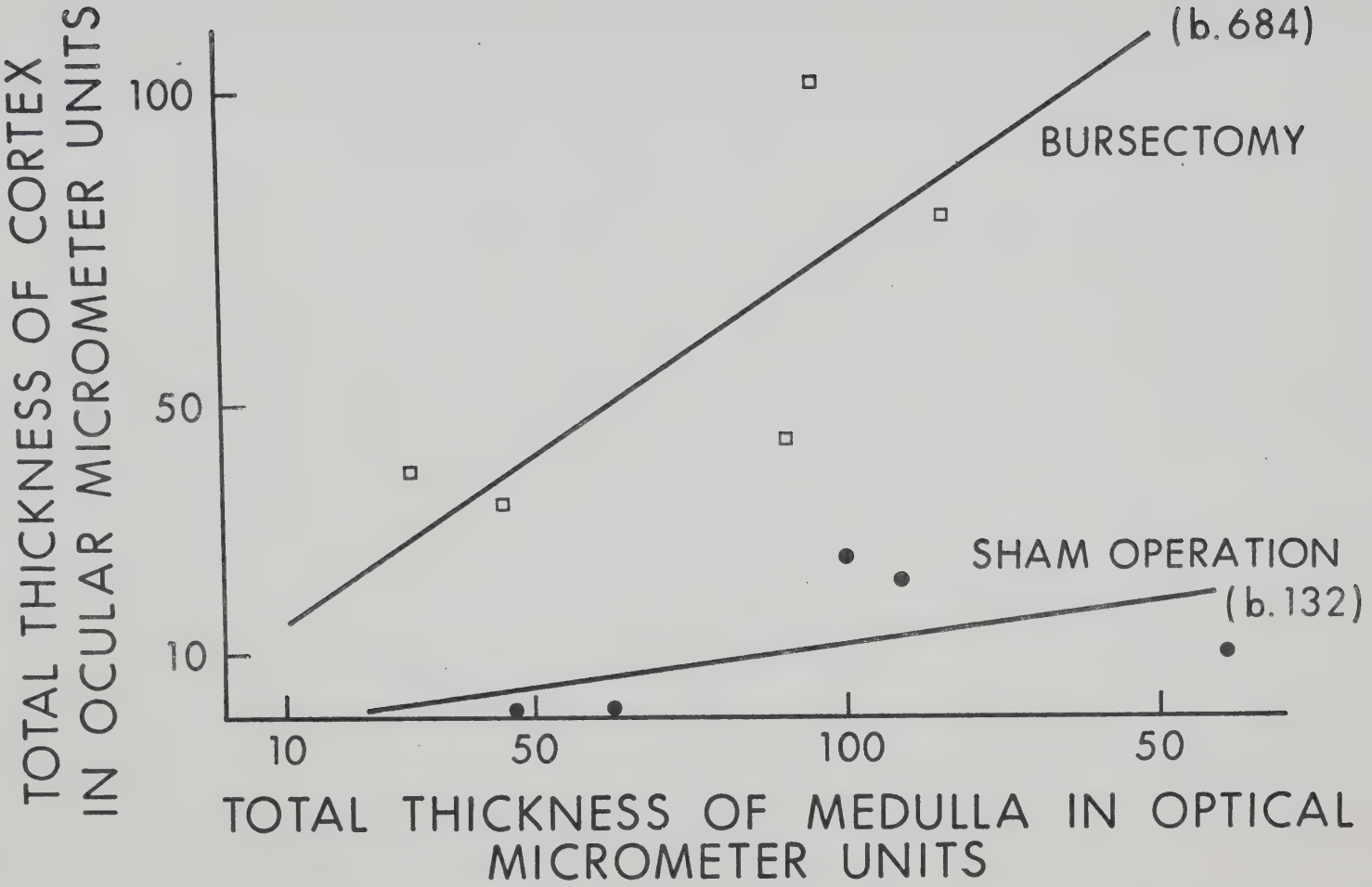

TOTAL LEUCOCYTES /cu. mm.×10³
80
70
60
50
40
30
0830
1630
2230
0830

Figure 53 - Relation of relative size of thymus cortex and medulla in female bursectomized or sham operated at fourteen weeks of age. At twenty weeks of age, histological preparations of sections of thymus lobes were measured in terms of ocular micrometer units to arrive at the relative thickness of the cortex and medulla. Each point represents the mean value of at least 10 sections of two thymus lobes from one individual bird. The lines were obtained from regression analysis of cortex thickness on medulla relative thickness. The correlation coefficients were bursectomy 0.77, control sham operated 0.46. The low numbers of individuals did not permit a test of significance. The slopes of the lines (b), were significantly different by t test. (t 3.266 **).


(b.684)
BURSECTOMY
SHAM OPERATION
(b.132)
100
50
10
10
50
100
50
TOTAL THICKNESS OF CORTEX IN OCULAR MICROMETER UNITS
TOTAL THICKNESS OF MEDULLA IN OPTICAL MICROMETER UNITS